



TRANSMITTAL LETTER FOR FILING
EXPRESS ABANDONMENT

Mail Stop Express Abandonment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir or Madam:

Please find included herewith for filing an Express Abandonment with respect to the patent application referenced below:

In re Patent Application of:
Cheryl R. Mitchell et al.

Application No.: 10/614,940

Confirmation No.: 9256

Filed: July 7, 2003

Art Unit: 1645

For: ORAL REHYDRATION COMPOSITIONS
CONTAINING LIPOSOMES

Examiner: Not Yet Assigned

Enclosed is:

- a) An Express Abandonment Under 37 CFR 1.138; and
- b) A Statement Under 37 CFR 3.73(b).

The enclosed Express Abandonment of the above-referenced application (the "Mitchell Application") is filed in part due to concerns of Phlo System, Inc. ("PSI"), the assignee of one hundred percent (100%) of the right, title and interest in the technology covered by the application, that:

- 1) The applicants intentionally omitted to include James B. Hovis as an inventor;
- 2) The applicants did not comply with their duty of disclosure under 37 CFR 1.56 et. seq.
 - a) For example, with respect to the less salty taste of the hydration beverage (referenced, e.g., on page 6 of the Mitchell Application), US Patent Application No. US 2002/0012689 A1 by Stillman, published in Jan. 31, 2002, discloses a hydration formula that can include encapsulated vitamins, minerals, and nutraceuticals (see paragraphs [0082]-[0088] and [0376]-[0389]. It teaches, among other things, encapsulation for taste

masking and discloses that the hydration beverage can be in a powder form and reconstituted with water prior to drinking (paragraph [0374]).

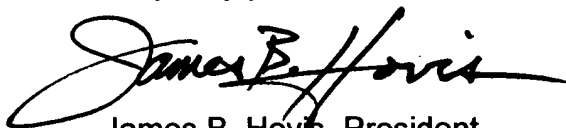
b) With respect to the Mitchell Application claims that "It was a surprising and unexpected result, based on results from a rat perfusion study, that the liposomed electrolytes were not only absorbed rapidly but apparently by a pathway in the digestive system other than the standard electrolyte absorption or by the method of a "nutrient assist" from either carbohydrates, proteins or amino acids." (Page 8 of the Mitchell Application). PSI is aware that this statement is blatantly false. PSI made inventor Cheryl R. Mitchell aware, on a confidential basis, of work performed by Robert G. Lamb, Ph.D. and on US Patent Application US 2005/0181021 A1 (which has now been granted but not yet issued) by Lamb (and licensed to PSI), including Declarations of Lamb in support of the application which are not yet part of the public record. This work leads one disciplined in the art to a thesis that electrolytes could be encapsulated in polyenylphosphatidylcholine liposomes to allow absorption of the electrolytes directly into the bloodstream without waiting for the digestive process to occur. Moreover, as the results of the referenced rat perfusion study sets forth, the study was performed with the goal of confirming the thesis of the rapid absorption of the beverage containing liposomed electrolytes; these were no "surprising and unexpected results".

c) PSI also believes that the applicants had a duty to disclose other prior art, including, without limitation, the following: (i) US Patent No. 5,032,411 by Stray-Gundersen (pertaining to the preferable osmolality of rehydration beverages) (col. 6, lines 29032); (ii) US Patent No. 5,269,979 by Fountain (e.g., related to the method of forming vehicles for encapsulating passenger molecules and the products of this process); and (iii) an article entitled "Encapsulation in the food industry: a review," by Gibbs, Kermasha, Alli, and Mulligan, 50 International Journal of Food Sciences and Nutrition pp. 213-224 (1999). Copies of all documents referenced herein are included herewith for the PTO's reference.

Pursuant to the provisions of 37 CFR 1.138, PSI respectfully requests that this application be abandoned.

Dated: January 24, 2006

Very truly yours,

A handwritten signature in black ink, appearing to read "James B. Hovis", with a stylized flourish at the end.

James B. Hovis, President
Phlo System, Inc.
1133 Connecticut Avenue, NW
Suite 200
Washington, DC 20036
(410-952-0009)



MANUAL OF PATENT EXAMINING PROCEDURE

PTO/SB/24 (06-04)

Approved for use through 07/31/2006. OMB 0851-0031

U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

EXPRESS ABANDONMENT UNDER 37 CFR 1.138

Fax directly to the Pre-Grant Publication Division at (703) 305-8568; or
mail to: Mail Stop Express Abandonment
Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450

Application Number	10/614,940
Filing Date	July 3, 2003
First Named Inventor	C.B. Mitchell
Art Unit	1645
Examiner Name	Not yet assigned
Attorney Docket Number	

Please check only one of boxes 1 or 2 below:

(If no box is checked, this paper will be treated as a request for express abandonment as of the filing date of this paper.)

1. ☒ **Express Abandonment**
I request that the above-identified application be expressly abandoned as of the filing date of this paper.
2. ☐ **Express Abandonment in Favor of a Continuing Application**
I request that the above-identified application be expressly abandoned as of the filing date accorded the continuing application filed previously or herewith.

NOTE: A paper requesting express abandonment of an application is not effective unless and until an appropriate USPTO official recognizes and acts on the paper. See the Manual of Patent Examining Procedure (MPEP), section 711.01.

TO AVOID PUBLICATION, PLEASE USE FORM PTO/SB/24A INSTEAD OF THIS FORM.

- I am the: ☐ applicant.
- ☒ assignee of record of the entire interest. See 37 CFR 3.71.
Statement under 37 CFR 3.73(b) is enclosed. (Form PTO/SB/96)
- ☐ attorney or agent of record. Registration Number _____
- ☐ attorney or agent acting under 37 CFR 1.34 (may act under 37 CFR 1.34 only if box 2 above, stating that the application is expressly abandoned in favor of a continuing application, is checked). Attorney or agent registration number if acting under 37 CFR 1.34. _____

(Attorney or agent registration number)

PhloSystem, Inc.

By: James B. Hovis
Signature

January 24, 2006
Date

James B. Hovis, President
Typed or printed name

410-952-0009
Telephone Number

Note: Signature of all the inventors or assignees of record of the entire interest or their representative(s) are required. Submit multiple forms if more than one signature is required, see below.

☐ Total of _____ forms are submitted.

This collection of information is required by 37 CFR 1.138. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process an application). Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. **DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Mail Stop Express Abandonment, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.



OWNERSHIP AND ASSIGNMENT

324

**>

PTO/SB/98 (09-04)

Approved for use through 07/31/2008. OMB 0651-0031

U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

STATEMENT UNDER 37 CFR 3.73(b)

Applicant/Patent Owner: Cheryl R. Mitchell and James B. MitchellApplication No./Patent No.: 101614,940 Filed/Issue Date: July 7, 2006

Entitled:

Phlo System, Inc. a Corporation
(Name of Assignee) (Type of Assignee, e.g., corporation, partnership, university, government agency, etc.)

states that it is:

1. ☒ the assignee of the entire right, title, and interest; or
2. ☐ an assignee of less than the entire right, title and interest.
The extent (by percentage) of its ownership interest is _____ %

in the patent application/patent identified above by virtue of either:

A. ☒ An assignment from the inventor(s) of the patent application/patent identified above. The assignment was recorded in the United States Patent and Trademark Office at Reel 015791, Frame 0045, or for which a copy thereof is attached.

OR

B. ☐ A chain of title from the inventor(s), of the patent application/patent identified above, to the current assignee as shown below:

1. From: _____ To: _____
The document was recorded in the United States Patent and Trademark Office at Reel _____, Frame _____, or for which a copy thereof is attached.
2. From: _____ To: _____
The document was recorded in the United States Patent and Trademark Office at Reel _____, Frame _____, or for which a copy thereof is attached.
3. From: _____ To: _____
The document was recorded in the United States Patent and Trademark Office at Reel _____, Frame _____, or for which a copy thereof is attached.

☐ Additional documents in the chain of title are listed on a supplemental sheet.☐ Copies of assignments or other documents in the chain of title are attached.

[NOTE: A separate copy (i.e., a true copy of the original assignment document(s)) must be submitted to Assignment Division in accordance with 37 CFR Part 3, if the assignment is to be recorded in the records of the USPTO. See MPEP 302.08]

The undersigned (whose title is supplied below) is authorized to act on behalf of the assignee.

James B. Hovis
SignatureJames B Hovis

Printed or Typed Name

President

Title

Jan. 24, 2006

Date

410-952-0009

Telephone Number

This collection of information is required by 37 CFR 3.73(b). The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

<

Report of a Study (27 June 2003, revised)

Absorption of water and electrolytes from a liposomal oral rehydration solution: an *in vivo* perfusion study of rat small intestine

Investigators: P.K. Bardhan, A.S.M. Hamidur Rahman, David A. Sack.

Report prepared by: David A. Sack, M.D., one of the world's foremost authorities on rehydration, who assisted Advanced Bio-Delivery, LLC in the evaluation and testing of the Aquis Liposomal ORS. Dr. Sack has been on the faculty of Johns Hopkins University in Baltimore, Maryland since 1975, including heading the Vaccine Testing Unit which, among other things, specializes in studying improvements to ORSs. Dr. Sack is also the Director of the International Center for Diarrheal Disease Research, Bangladesh (ICDDR), where his work led to the validation of a vaccine now recommended by the World Health Organization (WHO).

Summary. Aquis Liposomal ORS, a new oral rehydration solution containing complex carbohydrates and encapsulating the electrolyte payload for superior absorption, was recently evaluated by the ICDDR, a leading institute for oral rehydration research. Results indicated a clear superiority in water and electrolyte absorption as compared with other oral rehydration solutions, including the current advanced WHO formulation. Additionally, because the salts are micro-encapsulated in liposomes, Aquis Liposomal ORS tastes less salty and is much more acceptable to those drinking it.

The ICDDR is the institution that pioneered the discovery of oral rehydration solutions and has recently received the Gates Award for Global Health in recognition of this contribution. The scientists involved in the study include a scientist with much experience in the treatment of severe diarrhea patients and advanced training in gastroenterology (Dr. Bardhan), a veterinarian experienced in animal research studies with ORS (Dr. Rahman), and an authority on ORS from Johns Hopkins University who is experienced in the science and in clinical trials with ORS (Dr. Sack).

Background and rationale. Loss of fluids and electrolytes in patients with acute watery diarrhea leads to dehydration. The mainstay of treatment of such patients is fluid replacement either intravenously (i.v.) or by oral rehydration solution (ORS). The science and practice of oral rehydration has led to major advances in health during the last 20 years, and the World Health Organization (WHO) estimates that its use is now saving the lives of about 3 million children worldwide, compared to 20 years ago. While the use of ORS is relatively simple, the science behind its use depends on an understanding of the physiology of water and salt absorption from the intestine. If one uses the incorrect formulation of ORS, this can result in increased secretion and abnormalities of serum electrolytes, which can be serious and even fatal.

The principles of oral rehydration depend on defining the composition of the fluid being lost in abnormal quantities (e.g. diarrhea fluid or sweat) and using this as a guide to define the electrolyte concentrations in the ORS. The electrolyte concentrations in ORSs should approximate the electrolyte composition of the fluid that is being lost. However, the composition of the solution must be adjusted to the type of fluid being lost. For diarrhea losses, the solution should replace large amounts of sodium, potassium and base (e.g. citrate). For sweat losses, the solution should contain lesser amounts of these electrolytes, but their replacement is still critical for optimal physiologic homeostasis. Normally a diarrhea

replacement solution should contain 75 mmol of sodium, but for sweat losses, sodium should be about 20 mmol per liter. The other electrolytes should likewise be adjusted for the intended purpose of the solution.

Additionally, when ORSs are used, the electrolytes must be accompanied by a substrate (e.g. glucose) that facilitates the absorption of salts; otherwise the ORS will simply pass through the intestine, will not be absorbed, and will actually increase the amount of diarrheal losses. The composition of the ORS, in terms of appropriate electrolytes and substrate, is thus critical to achieve rehydration without inducing electrolyte abnormalities in the patient such as hyper or hyponatremia, hyper or hypokalemia, or persistent acidosis. The most commonly used substrate is glucose because it is effective, inexpensive and readily available. Other simple sugars (e.g. fructose) are not nearly as effective in the absorption of sodium.

The total osmolarity of the ORS should never exceed that of the blood since net absorption is dependent on both the substrate mediated absorption as well as osmotic forces. A hypertonic solution will pull fluid from the blood into the gut and decrease net absorption. This osmotic effect is known as the “osmotic penalty.” By contrast, a hypotonic solution will increase net absorption using these same osmotic forces. Since the goal of administering an ORS is to replace abnormal fluid and electrolyte losses as quickly as possible, this goal will be best accomplished with a hypotonic solution having the correct electrolytes that matches that which is being lost.

For patients with severe diarrhea, an ORS containing a complex carbohydrate is superior to one containing glucose, since complex carbohydrates provide more glucose molecules without adding to the overall osmolarity. If one were to increase the concentration of glucose in an attempt to increase the amount of absorption, the osmotic penalty would overcome the potential benefit. The superiority of complex carbohydrates has been shown in several clinical studies in patients with cholera, the most severe form of watery diarrhea. This benefit is summarized by the concept of “increased glucose carrying capacity without the osmotic penalty.” Based on these clinical studies showing the superiority when using a complex carbohydrate, the ICDDRb has been using a rice-based ORS since the early 1980's. While rice ORS has been used most commonly, complex carbohydrates from other sources (e.g. tapioca) can also be used.

Based on numerous clinical studies, WHO recommends a solution for treating dehydration resulting from diarrhea containing the following concentrations of electrolytes: sodium, 75 mmol/L; potassium, 20 mmol/L; citrate, 10 mmol/L. The substrate for this ORS may be either glucose (75 meq per liter) or another more complex carbohydrate.

A further advance in ORS technology is illustrated by the results of a set of experiments involving Aquis Liposomal ORS, manufactured by Advanced Bio-Delivery, LLC, an ORS which contains a complex carbohydrate and employs the micro-encapsulation of the electrolytes in liposomes. Recently, scientists at the ICDDRb have been conducting studies with Aquis Liposomal ORS, which in an animal model, demonstrated superior water and electrolyte absorption as compared to both the standard glucose-ORS (WHO-ORS) and a hydrolyzed starch ORS (prepared with partially hydrolyzed tapioca (a complex carbohydrate)) (HS-ORS). Aquis Liposomal ORS is prepared with a complex carbohydrate (partially hydrolyzed tapioca) and uses the same composition of electrolytes as recommended by WHO, but it also encapsulates the electrolytes in liposomes to add another mechanism of absorption. Liposomes can be incorporated into cell membranes and can provide a mechanism for salt and water absorption in addition to that provided by the glucose-mediated transport and the osmotic forces.

The studies comparing Aquis Liposomal ORS with WHO-ORS and HS-ORS use a rat model in which the entire small intestine is perfused with the test solution. Some groups of rats are control rats and other groups are rats whose intestinal mucosa has been stimulated with cholera toxin to induce fluid secretion. A third group of rats has been treated with 5-Fluorouracil to simulate the child with chronic diarrhea with blunted villi and poor absorption. In all groups of rats, we are able to measure the net absorption of the test solutions.

Experimental procedure. Adult male rats, weighing 250-300 g, were fasted for 24 hours with free access to water. The rats were then anaesthetized with intraperitoneal pentobarbital (40 mg/kg). The abdomen was then opened with a midline incision and the small intestine was cannulated with polyvinyl tubing. The proximal cannula was introduced into the distal stomach 3-4 cm proximal to the duodeno-jejunal flexure and gently guided into the duodenum through the pylorus. The distal cannula was inserted 3-4 cm proximal to the ileocaecal junction. The isolated and cannulated segment was then gently rinsed to clear of residual contents with the perfusion solution. Prior to the final wash, the intestine was returned to the abdominal cavity and the abdominal cavity was closed by suturing the incision and keeping the cannulas out of abdomen. The perfusion was started by attaching the proximal cannula to a constant infusion pump, at the constant rate of 0.5 ml/min. After 30 minutes of equilibration to achieve a steady state, the effluent was collected for 3 consecutive 15 minute-collections (total 45 minutes) in measuring cylinders kept on ice.

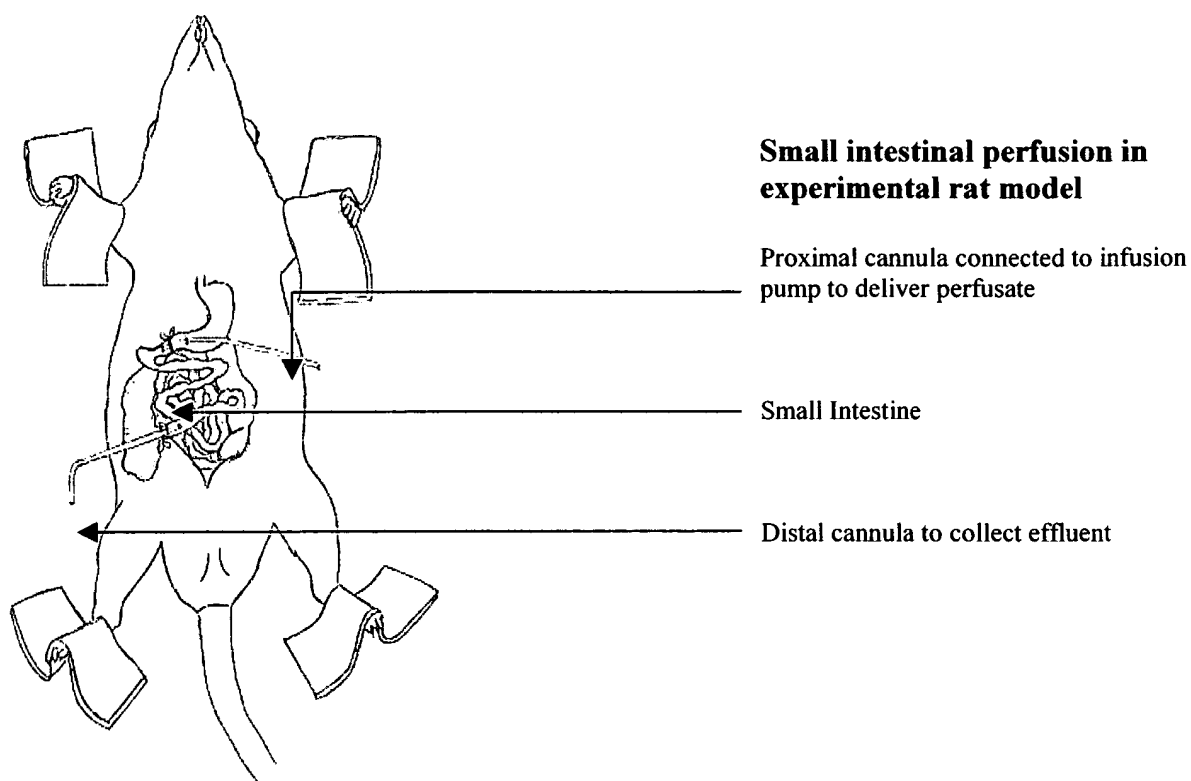
Each rat was perfused with one solution only - either the WHO-ORS, the HS-ORS, or the Aquis Liposomal ORS. Polyethylene glycol (PEG), MW 4,000, 2 g/L was used as the non-absorbable marker in order to calculate net absorption.

Three groups of experiments have been performed.

1. Comparison of absorption rates of WHO-ORS, HS-ORS, and Aquis Liposomal ORS in normal rat small intestine.
2. Comparison of absorption rates of WHO-ORS, HS-ORS and Aquis Liposomal ORS in rat small intestine after intestinal secretion is stimulated by cholera toxin (CT).
3. Comparison of absorption rates of WHO-ORS, HS-ORS and Aquis Liposomal ORS in rat small intestine after the rats were pre-treated with 5-Fluorouracil (5-FU). The 5-FU is a cytotoxic antimetabolite drug that causes damage to the intestinal mucosa thereby mimicking long-term compromised intestines. The 5-FU treated rats were included to simulate the child with chronic diarrhea with blunted villi and poor absorption.

For the CT experiment, a secretory state was induced before perfusion by the instillation of 75 µg of pure cholera toxin dissolved in 5 ml of isotonic sodium chloride solution via the distal cannula, and then the cannulas was clamped (Elliot, 1991). The CT-saline was evenly distributed within the entire small intestine, and the abdomen closed. The CT-saline solution remained in the small intestine for 2 hours to maximally stimulate intestinal secretion, after which the clamps on the cannulas were removed and the intestinal contents were allowed to drain out by gravity drainage. Then intestinal perfusion was started. The figure illustrates the methods of the rat experiments.

For each set of experiments, groups of rats were infused with either WHO-ORS, HS-ORS, or Aquis Liposomal ORS.



Results. The results of the studies of normal rats and rats that have been stimulated with cholera toxin using WHO-ORS, HS-ORS, and Aquis Liposomal ORS are shown on the table set forth below. As seen, all of the ORS solutions resulted in significant absorption, but the Aquis Liposomal ORS provided a significantly and statistically higher level of absorption in the normal and the cholera toxin stimulated rats as well as the 5-FU treated rats. In this model, there was essentially no difference observed between the WHO-ORS and the HS-ORS. Table 1 and 2 below show the results of the absorption experiments. Table 1 expresses the absorption as ml/minute/gram dry weight while Table 2 expresses the data from the same rats as ml/minute/cm length of intestine.

Table 1. Average water absorption in intestines of normal rats and intestines stimulated with cholera toxin and with 5-FU. (All values are expressed as mean absorption of water, ml/min/gram dry weight \pm SD)			
	WHO-ORS	HS-ORS	Aquis Liposomal ORS
Normal rats	0.231 \pm 0.053 (n=9)	0.247 \pm 0.08 [7%] (n=11)	0.323 \pm 0.01 [40%] (n=8)
Cholera toxin stimulated rats	0.224 \pm 0.045 (n=8)	0.225 \pm 0.038 [0%] (n=8)	0.326 \pm 0.011 [46%] (n=8)
5-FU treated rats	0.326 \pm 0.095 (n=12)	0.425 \pm 0.012 [30%] (n=10)	0.487 \pm 0.017 [49%] (n=9)

All values are expressed as mean \pm SD. [%] represents % increase compared to WHO-ORS.

Table 2. Average water absorption in intestines of normal rats and intestines stimulated with cholera toxin and with 5-FU. (All values are expressed as absorption of water, ml/min/cm \pm SD)			
	WHO-ORS	HS-ORS	Aquis Liposomal ORS
Normal rats	0.0038 \pm 0.0007 (n=9)	0.0039 \pm 0.0008 [3%] (n=11)	0.0047 \pm 0.0009 [24%] (n=8)
Cholera toxin stimulated rats	0.0042 \pm 0.0008 (n=8)	0.0041 \pm 0.0007 [2%] (n=8)	0.0055 \pm 0.001 [31%] (n=8)
5-FU treated rats	0.0056 \pm 0.0015 (n=12)	0.0067 \pm 0.0012 [20%] (n=10)	0.0089 \pm 0.0017 [59%] (n=9)

All values are expressed as mean \pm SD. [%] represents % increase compared to WHO-ORS.

Discussion. The study was planned to test the hypothesis that the use of Aquis Liposomal ORS would result in a higher absorption of salt and water. The results of the study are consistent with this hypothesis. The HS-ORS performed as well as the WHO-ORS and served as a control that suggests that the liposomes were responsible for the increased absorption found with the use of the Aquis Liposomal ORS. The results were consistent between the normal and the cholera toxin stimulated rats, and in fact the absorption was greater in the cholera toxin stimulated rats. The Aquis Liposomal ORS was also absorbed better in those rats that were pre-treated with 5-FU, suggesting that the liposomes are absorbed from damaged mucosa as well as from healthy mucosa. The greater absorption of the Aquis Liposomal ORS compared to the other two ORS solutions within each group of rats is highly statistically significant.

A practical improvement, in addition to the physiological improvement with Aquis Liposomal ORS, is the much-improved taste. Because the salts are incorporated into liposomes, the Aquis Liposomal ORS tastes less salty and more acceptable to those who are drinking it.

In summary, the rat studies to date demonstrate that all of the solutions are well absorbed, but that the Aquis Liposomal ORS was associated with the highest level of water and electrolyte absorption.

Method for making solvent dilution microcarriers

Abstract

A method for forming vehicles for encapsulating passenger molecules which have been named solvent dilution microcarriers (SDMCs), and the products of this process, are disclosed which allows for immediate or delayed formation of the encapsulating vehicles following creation of a shelf-stable formed solution by dissolution of amphipathic bilayer-forming materials, appropriate solvent, and the passenger molecule, addition of aqueous solution, and further addition of solvent. The SDMCs are organized from the formed solution by dilution into an aqueous system, aerosolization, or rehydration in situ.

Inventors: **Fountain; Michael W.** (Knoxville, TN)

Assignee: **Fountain Pharmaceuticals, Inc.** (Largo, FL)

Appl. No.: **882801**

Filed: **May 14, 1992**

Current U.S. Class:

264/4.6; 264/4.1; 424/450

Intern'l Class:

A61K 009/12; A61K 009/127; B01J 013/02

Field of Search:

424/450 264/4.1,4.6

References Cited [Referenced By]

U.S. Patent Documents			
<u>3639257</u>	Feb., 1972	Harbort	252/316.
<u>3691090</u>	Sep., 1972	Kitajima et al.	252/316.
<u>3804776</u>	Apr., 1974	Yazawa et al.	252/316.
<u>3943063</u>	Mar., 1976	Morishita et al.	252/316.
<u>3993754</u>	Nov., 1976	Rahman et al.	424/177.
<u>4078052</u>	Mar., 1978	Papahadjopoulos	424/36.
<u>4188447</u>	Feb., 1980	Ehlenz	428/310.
<u>4217344</u>	Aug., 1980	Vanlerberghe et al.	424/60.
<u>4298594</u>	Nov., 1981	Sears et al.	424/19.
<u>4308166</u>	Dec., 1981	Marchetti et al.	252/316.
<u>4356167</u>	Oct., 1982	Kelly	424/38.
<u>4377567</u>	Mar., 1983	Geho	424/1.
<u>4389330</u>	Jun., 1983	Tice et al.	427/213.
<u>4485054</u>	Nov., 1984	Mezei et al.	264/4.
<u>4505935</u>	Mar., 1985	Larsson	514/779.
<u>4508703</u>	Apr., 1985	Redziniak et al.	424/38.
<u>4515736</u>	May., 1985	Deamer	264/4.
<u>4522803</u>	Jun., 1985	Lenk et al.	424/1.
<u>4532123</u>	Jul., 1985	Gardner	264/4.
<u>4551288</u>	Nov., 1985	Kelly	264/4.
<u>4557935</u>	Dec., 1985	af Ekenstam et al.	424/130.
<u>4565696</u>	Jan., 1986	Heath et al.	424/88.
<u>4588578</u>	May., 1986	Fountain et al.	424/1.
<u>4599226</u>	Jul., 1986	Fox, Jr. et al.	424/27.
<u>4608211</u>	Aug., 1986	Handjani et al.	264/4.
<u>4610868</u>	Sep., 1986	Fountain et al.	424/1.
<u>4619794</u>	Oct., 1986	Hauser	264/4.
<u>4619795</u>	Oct., 1986	Cohen	264/4.
<u>4685911</u>	Aug., 1987	Konno et al.	604/897.
<u>4708861</u>	Nov., 1987	Popescu et al.	424/1.

<u>4717676</u>	Jan., 1988	Wagner et al.	436/501.
<u>4721612</u>	Jan., 1988	Janoff et al.	424/1.
<u>4731210</u>	Mar., 1988	Weder et al.	264/4.
<u>4737323</u>	Apr., 1988	Martin et al.	264/4.
<u>4752572</u>	Jun., 1988	Sundberg et al.	435/7.
<u>4755388</u>	Jul., 1988	Heath et al.	424/450.
<u>4769028</u>	Sep., 1988	Hoffmann et al.	424/443.
<u>4783402</u>	Nov., 1988	Kokusho et al.	435/52.
<u>4797284</u>	Jan., 1989	Loper et al.	424/449.
<u>4804539</u>	Feb., 1989	Guo et al.	424/450.
<u>4808402</u>	Feb., 1989	Leibovich et al.	424/423.
<u>4810499</u>	Mar., 1989	Nuwayser	424/448.
<u>4812407</u>	Mar., 1989	Buchmann et al.	435/291.
<u>4839175</u>	Jun., 1989	Guo et al.	424/450.
<u>4921757</u>	May., 1990	Wheatley et al.	428/402.
<u>4959341</u>	Sep., 1990	Wallach	502/404.
<u>4975421</u>	Dec., 1990	Williams et al.	514/54.
<u>5000958</u>	Mar., 1991	Fountain et al.	424/450.
<u>5141674</u>	Aug., 1992	Leigh	424/450.
<u>5154930</u>	Oct., 1992	Popescu et al.	424/450.

Foreign Patent Documents

0158441	Oct., 1985	EP.
0186542	Jul., 1986	EP.
0249561	Dec., 1987	EP.
8500515	Feb., 1985	WO.
8804573	Jun., 1988	WO.
8901790	Mar., 1989	WO.

Other References

- Derwent Abstract, 86-171194/27 (corresponding to EP 0186542).
- A. Bangham, M. Standish, and J. Watkins, "Diffusion of Univalent Ions Across the Lamellae of Swollen Phospholipids," 13 J. Mol. Biol., 238-252 (1965) (United Kingdom).
- S. Batzri and E. Korn, "Single Bilayer Liposomes Prepared Without Sonication," 298 Biochimica et Biophysica Acta 1015-1019 (1973) (The Netherlands).
- J. Brunner, P. Skrabal, and H. Hauser, "Single Bilayer Vesicles Prepared Without Sonication Physico-Chemical Properties," 455 Biochimica et Biophysica Acta 322-331 (1976) (The Netherlands).
- Z. Chowhan, T. Yotsuyanagi, and W. Higuchi, "Model Transport Studies Utilizing Lecithin Spherules," 266 Biochim. Biophys. Acta 320-342 (1972) (The Netherlands).
- D. Deamer and A. Bangham, "Large Volume Liposomes By An Ether Vaporization Method," 443 Biochimica et Biophysica Acta 629-634 (1976) (The Netherlands).
- M. Mezei and V. Gulasekharam, "Liposomes-A Selective Drug Delivery System For the Topical Route of Administration," 26 Life Sciences 1473-1477 (1980) (United States).
- F. Szoka, Jr. and D. Papahadjopoulos, "Comparative Properties and Methods of Preparation of Lipid Vesicles (Liposomes)," 9 Ann. Rev. Biophys. Bioeng. 467-508 (1980) (Palo Alto, Calif., USA).
- D. Papahadjopoulos and J. Watkins, "Phospholipid Model Membranes-II. Permeability Properties of Hydrated Liquid Crystals," 135 Biochim. Biophys. Acta 639-652 (1967) (The Netherlands).
- D. Papahadjopoulos and N. Miller, "Phospholipid Model Membranes-I. Structural Characteristics of Hydrated Liquid Crystals," 135 Biochim. Biophys. Acta, 624-638 (1967) (The Netherlands).
- D. Papahadjopoulos, W. Vail, K. Jacobson, and G. Poste, "Cochleate Lipid Cylinders: Formation by Fusion of Unilamellar Lipid Vesicles" 394 Biochimica et Biophysica Acta 438-491 (1975).
- F. Szoka, Jr. and D. Papahadjopoulos, "Procedure for preparation of Liposomes with Large Internal Aqueous Space and High Capture by Reverse-Phase Evaporation," 75 Proc. Natl. Acad. Sci. USA 9 pp. 4194-4198 (Sep., 1978) (United States).
- O. Zumbuehl and Hans George Weder, "Liposomes of Controllable Size in the Range of 40 to 180 nm by Defined Dialysis of Lipid/Detergent Mixed Micelles," 640 Biochimica et Biophysica Acta 252-262

(1981) (The Netherlands).

Primary Examiner: Stoll; Robert L.

Assistant Examiner: Metzmaier; Daniel S.

Attorney, Agent or Firm: Richards, Medlock & Andrews

Parent Case Text

RELATED APPLICATION

This is a continuation of application Serial No. 460,838 filed Jun. 8, 1989 now U.S. Pat. No. 5,133,965, which is a continuation-in-part of application Ser. No. 204,214 filed Jun. 8, 1988 (now abandoned).

Claims

I claim:

1. A method for making a solvent dilution microcarrier vehicle comprising the steps of:
 - (a) solubilizing an amphipathic material and a passenger molecule in a first quantity of a non-aqueous solvent appropriate to solubilize both the amphipathic material and the passenger molecule to form a first solution;
 - (b) adding a first quantity of water to said first solution to form a turbid suspension;
 - (c) adding a second quantity of an appropriate non-aqueous solvent to said turbid suspension in a sufficient amount to cause a second solution to form, said second solution characterized by having optical clarity at room temperature and being monophasic at room temperature; and
 - (d) mixing said second solution with air or a second quantity of water sufficient to organize said second solution into a plurality of solvent dilution microcarrier vehicles encapsulating said passenger molecule, each of said solvent dilution microcarrier vehicles in said plurality being of substantially the same size.
2. The method of claim 1, wherein said first quantity of water is from about 4:1 (first quantity of non-aqueous solvent to first quantity of water) to about 10:1 (first quantity of non-aqueous solvent to first quantity of water).
3. The method of claim 1 wherein said second quantity of non-aqueous solvent is from about 15:1 (second quantity of non-aqueous solvent to first quantity of water) to about 100:1 (second quantity of non-aqueous solvent to first quantity of water).
4. The method of claim 1 wherein said passenger molecule is selected from the group consisting of antimicrobials, anti-inflammatories, anti-parasitics, dyes, radio labels, radio-opaque compounds, fluorescent compounds, immunomodulating compounds, peptides, proteins, glycoproteins, lipoproteins, hormones, neurotransmitters, tumorocidal agents, growth factors, toxins, analgesics, anesthetics, monosaccharides, polysaccharides, narcotics, catalysts and enzymes.
5. The method of claim 1 wherein said passenger molecule is lipid-soluble.
6. The method of claim 1 wherein said amphipathic material comprises at least one phospholipid.
7. The method of claim 1, wherein said second solution is mixed with air by aerosolizing.
8. The method of claim 1, wherein said second solution is mixed with water by adding a quantity of said second solution to water thereby causing immediate formation of solvent dilution microcarrier vehicles.

9. The method of claims 1, 7 or 8, wherein said plurality contains solvent dilution microcarrier vehicles ranging from 100 to 300 nanometers in diameter.

10. A method for making a solvent dilution microcarrier vehicle comprising the steps of:

(a) solubilizing an amphipathic material and a passenger molecule in a first quantity of a non-aqueous solvent appropriate to solubilize both the amphipathic material and the passenger molecule to form a first solution;

(b) adding a quantity of water to said first solution to form a turbid suspension;

(c) adding a second quantity of an appropriate non-aqueous solvent to said turbid suspension in a sufficient amount to cause a second solution to form, said second solution characterized by having optical clarity at room temperature and being monophasic at room temperature;

(d) applying said second solution to a surface and allowing said second solution to dry on said surface; and

(e) rehydrating said surface to form solvent dilution microcarrier vehicles.

11. A method for making a solvent dilution microcarrier vehicle comprising the steps of:

(a) solubilizing an amphipathic material and a passenger molecule in a first quantity of a non-aqueous solvent to form a first solution;

(b) adding a first quantity of water to said first solution to form a turbid suspension;

(c) adding a second quantity of an appropriate non-aqueous solvent to said turbid suspension in a sufficient amount to cause a second solution to form, said second solution characterized by having optical clarity at room temperature and being monophasic at room temperature; and

(d) diluting an aliquot of said second solution in a second quantity of water in a ratio from about 1:5 to about 1:100 to form solvent dilution microcarrier vehicles.

12. The method according to claim 11 wherein said second solution is diluted in a ratio of from about 1 to about 10.

13. A method for forming a solvent dilution microcarrier vehicle comprising the steps of:

(a) solubilizing an amphipathic material and a passenger molecule in a first quantity of a non-aqueous solvent appropriate to solubilize both the amphipathic material and the passenger molecule to form a first solution;

(b) adding a quantity of water to said first solution to form a turbid suspension;

(c) adding a second quantity of an appropriate non-aqueous solvent to said turbid suspension in a sufficient amount to cause a second solution to form, said second solution characterized by having optical clarity at room temperature and being monophasic at room temperature; and

(d) aerosolizing said second solution by mixing it with air to form solvent dilution microcarrier vehicles.

14. The method according to claim 13 wherein said second solution is mixed with air by spraying through a conventional sprayer.

Description

TECHNICAL FIELD

This invention relates to the art of enclosing passenger molecules in a amphipathic carrier structure.

BACKGROUND OF THE INVENTION

There have been numerous attempts in the prior art to develop lipid-based vesicles which are capable of entrapping various materials of interest ("passenger molecules"). The known methods have generally resulted in generally spherical vesicles known as liposomes which are composed of a lipid bilayer having an inner space in which the entrapped material is held. These vesicles have been formed by methods employing mechanical agitation, for example, sonication or extrusion. After lipids in organic solvents were mixed, the resulting mixture was dried, followed by mechanical agitation and rehydration with the passenger molecule to be entrapped to encourage the lipid bilayer to enclose around the passenger molecule.

The liposomes formed by this method were generally heterogeneous in size and difficult to sterilize for in vivo applications. The stability or shelf-life of these liposomes was often very limited. The entrapment efficiency of passenger molecules was generally limited. The methods required, in general, toxic nonbiocompatible solvents. The prior procedures were not applicable to aerosolization or formation of liposomes in situ. The vehicles formed by this method generally could be sterilized only by filtration as they exhibited heat lability. Moreover, prior methodology was not acceptably adaptable to the entrapment of certain passenger molecules.

SUMMARY OF THE INVENTION

It has now been found that an amphipathic vehicle (hereinafter referred to as "Solvent Dilution MicroCarriers" or "SDMCs") can be made using a method which leads to entrapping the passenger molecule in the bilayer itself, or in association with a component of the bilayer, rather than inside the space created by a spherical bilayer. In this method, a solvent is mixed with an amphipathic material and a passenger molecule. A small amount of water is then added to form a turbid solution. Additional solvent is then added to form an optically clear solution, also referred to as the "formed solution." An organization step is performed either immediately or at a time remote from the previous steps. The organization may involve aerosolization, dilution into aqueous materials or drying and rehydrating. The amphipathic vehicles or solvent dilution microcarriers (SDMCs) are formed upon employment of the organization method. The vehicles formed by this method exhibit substantial size homogeneity and are capable of being sterilized by sterile filtration, heating or u.v. irradiation.

The process may be put on hold after the formed solution is made and that solution held until it is desired to perform the organization step.

The SDMCs formed by this new method are unique in that they entrap a wide range of passenger molecules. The SDMCs have a wide range of applications. Aerosols containing SDMCs may be advantageously applied to large surface areas, such as for example when the passenger molecule is a pesticide. On the other hand, aerosols are also applicable to delivery of medicaments and cosmetics such as antibiotics or hair sprays. The methodology of this invention is also useful for SDMC formation in situ. The formed solution or SDMCs may be adsorbed on a surface such as bandage material and dried. Hydration allows the SDMC to deliver the passenger molecule to the desired site.

A sustained-release wound dressing material comprised of a foam bandage material and SDMCs has also been found.

DETAILED DESCRIPTION OF THE INVENTION

In the examples which follow, Solvent Dilution Microcarriers (SDMCs) were prepared by solubilizing at least one amphipathic material such as a surfactant (for example a biocompatible surfactant with good miscibility such as, but not limited to, Tween, Triton, sodium dodecyl sulfate (SDS), sodium laurel sulfate, and sodium octyl glucoside), a phospholipid, a mixture of phospholipids, polar lipids, sterols, sterol esters, neutral lipids, fatty acids, or other bilayer forming material into an appropriate solvent along with a passenger molecule (a material intended to solubilize in SDMCs). Various mixtures of amphipathic materials may be used. In some cases, it is convenient to select a commercial preparation of phospholipids. A preparation comprising about 75-97% mixed soy phosphatides may be used. For examples, commercial preparations of 75-80% mixed soy phosphatides, the remaining percentage being soy oils and preparations comprising 95-97% mixed soy phosphatides, the remaining percentage being soy oils are available. Commercial preparations having these characteristics are sold, for example, under the trade designations "Alcolec S", "Alcolec X-tra A" and "Alcolec LKE." The appropriate solvent is selected from those able to solubilize both the amphipathic material(s) and the passenger molecules. Generally, the most preferable solvent is

a low molecular weight hydrocarbon such as ethanol, propanol, butanol, isopropanol, chloroform, acetone, methylene chloride or propyl glycol and the like. In addition, the solvent must be appropriate for the particular intended use of the SDMCs. If the SDMCs are to be employed in vivo such as for example in an intravenous admixture (i.v. admixture), the solvent must be utilizable without causing toxicity in that application and generally must be biocompatible and readily miscible. In other applications, such as pesticide applications, the toxicity of the solvent is not as critical, but volatility becomes of more import. It is desirable in such case that the solvent volatilize immediately upon aerosolization. For formation in situ, such as for example when bandage material is impregnated for later rehydration, it is important that the solvent be non-flammable-, that it volatilize quickly, and leave no residue in the matrix. Methylene chloride is one example of an appropriate solvent for such an application. Mixtures of solvents may be appropriate in some circumstances. The passenger molecule may be generally any material capable of being retained in a formed bilayer or associated with that bilayer. Passenger molecules such as antimicrobials, antiinflammatories, anti-parasitics, dyes, radiolabels, radio-opaque compounds, fluorescent compounds, immunomodulating compounds, peptides, proteins, glycoproteins, lipoproteins, hormones, neurotransmitters, tumorocidal agents, growth factors, toxins, analgesics, anesthetics, mono and polysaccharides, narcotics, catalysts and enzymes are examples of the classes of substances which may be utilized. It is most preferred that the passenger molecule be lipophilic, however hydrophilic materials may also be utilized if they are capable of forming an association with the bilayer (i.e., the polar head group of the lipids). Cosmetics or cosmetic ingredients such as hair sprays, colorants, dyes, and the like are often highly appropriate for encapsulation in an SDMC. Medicaments used in mouthwashes, throat sprays, antiseptic sprays and the like also may be candidates for SDMC encapsulation. Drugs or tumorocidal agents for i.v. admixture or other introduction method may be encapsulated in an SDMC utilizing the disclosed method. It is envisioned that SDMC vehicles containing toxic drugs may be effectively administered to a patient by coupling the SDMC to site-specific monoclonal antibodies, for example. In the alternative, the encapsulation alone may allow administration of certain drugs that could not be administered in unencapsulated form in an efficient or effective manner.

Following the mixing of the bilayer-forming material, passenger molecule, and solvent, water is added in the ratio of from about 4:1 (solvent to water) to about 10:1 (solvent to water), to form a turbid solution. Additional solvent is then added in the ratio from about 15:1 (solvent to water) to about 100:1 (solvent to water) or until the formed solution is optically clear. The additional solvent will generally be the same as used in the first step, but it may be a mixture of solvents or a different solvent which is appropriate to accomplish the desired result.

An organization step is done either immediately, or after storage of the formed solution for an indefinite period. The organization step may be aerosolization, dilution into an aqueous solution or drying and rehydrating. Aerosolization is performed simply by putting the material formed as described above into a sprayer or trigger pump such as would be commonly used for applying non-pressurized hair sprays, insecticides, and the like to other surfaces. Upon spraying the formed solution, it is mixed with air and the volatile solvent evaporates as the solution leaves the nozzle.

The dilution method of organization comprises diluting an aliquot of formed solution into water. Upon dilution, the SDMCs are formed. One preferred appropriate dilution ratio is 1 to 10 (from 1 part formed solution to 9 parts water). The dilution ratio may range from about 1 to 5 to about 1 to 100.

The drying and rehydrating form of organization may be done by putting the formed solution onto a surface such as a bandage gauze, tampon, foam dressing, contraceptive sponge and the like and allowing the solvent to evaporate off very quickly. Upon hydrating the impregnated- gauze, the SDMCs are formed in situ and can perform their function of transporting the passenger molecule to the appropriate site on a wound, for example.

The SDMC vehicles formed by this method may be evaluated by utilizing a light scattering technique to determine the presence of vesicles. This technique can also be used to estimate the size of the SDMCs. Various instruments are commercially available for the sizing and counting of cells or other particles. The Coulter particle analyzers available from Coulter Electronics, Hialeah, Fla. have been found useful in this regard. Specifically the Coulter NM4AM multi-angle submicron particle analyzer has been successfully employed. Vehicle size may also be estimated using standard column chromatography techniques. The SDMCs have also been analyzed by testing the efficacy of the SDMC preparation over standard commercial preparations of the passenger molecule. The SDMCs have been found to have successfully encapsulated the molecule of interest by utilizing standard tests for the efficacy of the passenger molecule. For example, pesticides may be tested for efficacy in killing insects and antibiotics for efficacy in standard microbiological assays.

It has been found that SDMCs exhibit substantial size homogeneity. The size is believed to be dependent on the passenger molecule and identity of the amphipathic materials utilized, but it has been demonstrated that within one preparation of SDMCs, the size range is very compact. This characteristic is believed to be important in several applications of SDMCs including in vivo drug delivery.

The SDMCs have also been tested to be stable to heat sterilization and cobalt irradiation sterilization, which widens their utility for uses where sterility is required.

The optically clear solution is shelf-stable for months which allows one to delay SDMC formation until a later date. The organization step may be performed by the end user in many applications such as pesticide application by aerosolization, among others.

A sustained-release wound dressing is disclosed which utilized SDMCs in a foam bandage material. The foam bandage material suitable for the invention is preferably a crosslinked foam consisting of a mixture of polyolefins and other polymers (usually aryl-containing polymers) to form a three-dimensional network. Examples of commercially available forms of this material are Hypol.RTM. FMP 2002, marketed by W.R. Grace & Company (Lexington, Mass. 02173), and the Kontour sterile sponge, marketed by Winfield Laboratories, Inc. (P.O. Box 832616, Richardson, Tex. 75081). Other foam bandage material may be used as long as it is capable of loading the SDMCs and nontoxic to animals at the wound site and are preferably hydrophili, although they may be hydrophobic.

A method of wound treatment utilizing the sustained-release wound dressing is suitable for treating wounds that require at least about 30 minutes of therapy up to about seven days. The medicament is released slowly over the treatment time. A nonadherent dressing material may be applied to the wound prior to the foam bandage material.

The non-adherent dressing should be made from biocompatible synthetic or naturally occurring fibers arranged in a matrix having pores of a size large enough to allow the SDMCs to pass from the foam dressing to the wound site. Such wound dressings are available commercially from several companies. An example of this type of dressing is N-Terface.RTM. interpositional surfacing material which is available from Winfield Laboratories, Inc. in Richardson, Tex. 75083.

The following examples are intended to illustrate the invention, but is to be understood that various modifications thereof will be apparent to one skilled in the art and it is intended to cover such modifications as fall within the scope of the appended claims and that the examples are submitted for the purpose of providing a better understanding of the present invention and are not to be construed as limiting the scope thereof.

EXAMPLE 1

SDMCs Containing Gentamicin

A sample of 200 mg of soy lecithin was solubilized at room temperature with 20 mg of gentamicin base in 5 ml of absolute ethanol. Two ml of water was added to the solution which resulted in a turbid suspension. Six ml of absolute ethanol was added to the suspension yielding an optically clear solution. SDMCs were formed by dilution of 1 ml of final solution into 10 ml of aqueous solution. The resulting opalescent suspension of SDMCs was characterized by chromatographic separation over a Sepharose CL 4B column. The SDMCs were found to entrap virtually 100% of the starting gentamicin base as determined by a generally accepted bioassay of SDMCs containing gentamicin base to inhibit the growth of E. coli.

EXAMPLE 2

SDMCs Containing Vitamin A

A sample of 1100 mg of soy lecithin was solubilized at room temperature with 10 mg of retinol (vitamin A) in 2.5 ml of absolute ethanol. One ml of water was added to the solution which resulted in a turbid (cloudy) suspension. Three ml of absolute ethanol was added to the suspension yielding an optically clear solution. SDMCs were formed by dilution of 1 ml of final solution into 10 ml of aqueous solution.

EXAMPLE 3

SDMCs Containing Vitamin E

The procedure in Example 2 was followed except that vitamin A was replaced with vitamin E (.alpha.-tocopherol).

EXAMPLE 4

SDMCs Containing Vitamin D2

The procedure in Example 2 was followed except that vitamin A was replaced with vitamin D2 (ergocalciferol).

EXAMPLE 5

SDMCs Containing Vitamin D3

The procedure in Example 2 was followed except that vitamin A was replaced with vitamin D3 (cholecalciferol).

EXAMPLE 6

SDMCs Containing Vitamin K

The procedure in Example 2 was followed except that vitamin A was replaced with vitamin K (2-methyl-3 phytyl-1,4-naphthoquinone, 3 phytylmenadione).

EXAMPLE 7

SDMCs Containing Sterols

The procedure in Example 2 was followed except that vitamin A was replaced with sterol (cholesterol).

EXAMPLE 8

SDMCs Containing Pesticides

A sample 1 g of soy lecithin was solubilized into a 300 ml solution (petroleum distillate) containing 0.2% w/v mixed pyrethrins and 2.0% w/v piperonyl butoxide. Twenty ml of water was added to the solution which resulted in a turbid (cloudy) suspension. To the suspension was added 300 ml of petroleum distillates which resulted in an optically clear solution. SDMCs were formed by aerosolization of solution into air using a trigger pump and by dilution of 1 ml of solution into 10 ml of water. The efficacy of these preparations was demonstrated by their ability to kill fleas, ants, and paper wasps when SDMCs were aerosolized using a trigger sprayer or when the solution was directly applied onto the insects.

EXAMPLE 9

SDMCs as Animal Dips

The procedure in Example 8 was followed except that piperonyl butoxide and pyrethrins were replaced with malathion. The resulting solution upon dilution (200 ml into 70 liters of water) resulted in a turbid solution which was used as a flea dip for dogs.

EXAMPLE 10

SDMCs as Plant Insecticide Sprays

The procedure in Example 8 was followed except that the starting concentration of piperonyl butoxide was 0.2% w/v and mixed pyrethrins 0.02% w/v. The resulting solution was proven to be insecticidal and did not injure plant leaves upon aerosolization to form SDMCs.

EXAMPLE 11

SDMCs Containing Peppermint Oil

A sample of 200 mg of soy lecithin was solubilized in 5 ml of peppermint oil (1% w/v solution) in absolute ethanol. One-half ml of water was added to solution which became turbid. The turbid suspension was solubilized by addition of 5 ml of absolute ethanol. Upon dilution 1:10 to water, an opalescent suspension of SDMCs resulted. The entrapment of peppermint oil was demonstrated by a taste test evaluation comparing the SDMCs to peppermint oil in solvent alone. The evaluation demonstrated a prolonged peppermint taste resulted from the use of SDMCs as compared to peppermint oil in solvent alone.

EXAMPLE 12

SDMCs Containing Vanilla

The procedure in Example 11 was followed except that peppermint oil was replaced by vanilla.

EXAMPLE 13

SDMCs Containing Pineapple Oil

The procedure in Example 11 was followed except that peppermint oil was replaced by pineapple oil.

EXAMPLE 14

SDMCs Containing Anise Oil

The procedure in Example 11 was followed except that peppermint oil was replaced by anise oil.

EXAMPLE 15

SDMCs Containing Orange Extract

The procedure in Example 11 was followed except that peppermint oil was replaced by orange extract.

EXAMPLE 16

SDMCs Containing Facial Cleansers

A sample of 200 mg of soy lecithin was solubilized with 10% w/v salicylic acid in 10 ml isopropyl alcohol. One ml of water was added to form a cloudy turbid suspension. Ten ml of isopropyl alcohol was added to the suspension which resulted in an optically clear solution. The solution (1 ml) was diluted into 10 ml of water resulting an opalescent suspension of SDMCs. The material was tested and found to demonstrate good contact time on skin surfaces.

EXAMPLE 17

SDMCs Containing Fragrances

Ten ml of alcohol-based perfume was solubilized with 200 mg of soy lecithin. One ml of water was added to solution resulting in a cloudy suspension. The suspension was solubilized by addition of 10 ml of absolute ethanol. SDMCs were formed by aerosolization using a trigger pump and by dilution 1:10 w/v in water. The SDMCs were tested by application to skin. It was found that fragrances could be detected by olfaction longer than standard preparations were so detectable.

EXAMPLE 18

SDMCs in Mouthwash

A sample of 300 mg of soy lecithin was solubilized in 30 ml of 70% w/v alcohol 38B containing 1.0% of oil of peppermint w/v. Three ml of water was added to the solution which resulted in a cloudy suspension. Thirty ml of 70% v/v alcohol 38B was added to suspension to form a solution. The mouthwash was tested by dilution in saliva upon gargling and by prior dilution 1:10 v/v of solution into water prior to gargling. The results demonstrated prolonged contact of SDMCs in mouth by taste of peppermint oil SDMCs when compared with mouthwash lacking SDMCs.

EXAMPLE 19

SDMCs as Hair Spray

To a 20 ml sample of a commercially available liquid hair spray was added 200 mg of soy lecithin. To the solution was added 2 ml of water resulting in a cloudy suspension. Twenty ml of commercially available liquid hair spray was added to suspension resulting in an optically clear solution. The solution was aerosolized using a finger pump and found effective as a hair spray.

EXAMPLE 20

Stability of SDMC Forming Solution

An optically clear solution formed as describe in Example 8 was stored at room temperature for 15 months and demonstrated an optically clear solution which, upon aerosolization or dilution into water resulted in an opalescent SDMC suspension which was active as an insecticide in killing ants, fleas, and paper wasps.

EXAMPLE 21

SDMCs in A Gauze Pad Dressing

The procedure in Example 1 was followed resulting in a clear solution which, upon dilution, formed an opalescent suspension of SDMCs entrapping gentamicin base. The suspension was aerosolized onto gauze pads. SDMCs formed on the surface and were allowed to dry. After drying, the bandage material was tested in bioassays using generally accepted laboratory procedures and it was found that there was active gentamicin base. The bandage material onto which SDMCs were aerosolized was saturated with water and allowed to stand. The water was removed and yielded an opalescent suspension of SDMCs which contained active gentamicin base when tested by bioassay with *E. coli*.

EXAMPLE 22

Antimicrobial SDMCs in Tampons

The procedure would be the same as employed in Example 21, except that tampons would be used rather than gauze pads.

EXAMPLE 23

Entrapment of PCMX (Poly-Chloro-Meta-Xylenol)

To 185 ml of ethanol was added 18.5 ml of water. 295 ml. of Tween detergent was added to ethanol/water mixture. 50 ml soy lecithin was added to the above solution. 165 grams of PCMX was added and stirred to form a yellow clear solution. The clear yellow PCMX solution was diluted 1:10 in water. This dilution yielded an optically turbid solution of solvent dilution microcarriers containing solubilized PCMX.

EXAMPLE 24

Antimicrobial Mouthwash

To 200 ml of a 70% ethanol-containing commercial mouthwash base was added a solution containing 45 ml of ethanol, 20 grams of soy lecithin, 0.5 grams of PCMX and 5 ml of water. The solutions were mixed and yielded a

final solution containing 0.2% PCMX. The antimicrobial activity of the resulting mouthwash was tested and found to be increased over both commercial mouthwash and mouthwash lacking SDMCs but containing PCMX.

EXAMPLE 25

Intravenous Admixture for Tetrachlordecaoxid

An intravenous solution for injection (i.v. admixture) was prepared. Into 50 ml of ethanol was solubilized 6.9.times.10.sup.8 I.U. of Tetrachlordecaoxid (TCDO) and 5 grams of soy lecithin. Water (5 ml) was added to the ethanol solution to form a turbid solution. 50 ml of ethanol was added to clarify the turbid solution. SDMCs were prepared by dripping TCDO solution into 1 liter of 0.9% w/v saline. The SDMCs which resulted by dispersion into saline were analyzed by using light scattering to detect the presence of the vesicles. A Coulter NM4AM multi-angle submicron particle analyzer (Coulter Electronics, Hialeah, Fla.) was utilized according to the manufacturer's instructions for the light scattering technique. Antimicrobial activity was accessed and found to be present at least 4 days after dilution using generally accepted laboratory techniques with E. coli.

EXAMPLE 2

Gentamicin In An i.v. Admixture

An i.v. admixture of gentamicin was prepared as described in Example 25 by substituting 0.1 grams gentamicin for TCDO.

EXAMPLE 27

Gentamicin SDMC's in Foam Wound Dressing

To a solution of 50 ml of methylene chloride was added 10 grams of soy lecithin, 0.1 grams of gentamicin base and 5 ml of water. 50 ml of additional methylene chloride was added. Onto a commercial surgical foam wound bandage was poured the above solution and the methylene chloride allowed to evaporate. The sponge material containing the SDMC forming solution was mixed with water and evaluated using light scatter as described in Example 25. The size of SDMC's which emerged from foam material was from about 190 to 200 nm (mean size 180). The foam material with SDMC forming solution was tested in bioassays using E. coli and it was found that there was active gentamicin base as determined by standard antimicrobial test procedures.

EXAMPLE 28

Silver Sulfadiazene SDMC's in Foam Wound Dressing

The procedure as in Example 27 was repeated with the exception that silver sulfadiazene (1% w/w) was added in place of gentamicin base. The mean particle size was found to be about 210 nm.

EXAMPLE 29

TCDO in a Foam Wound Dressing

6.9.times.10.sup.7 I.U. TCDO was impregnated into foam wound dressing as described for gentamicin in Example 27. The mean size was found to be 220 nm.

EXAMPLE 30

SDMC containing Methoprene in Foam Wound Dressing

The procedures in Example 27 was repeated with exception that methoprene (0.2% w/w) replaced gentamicin.

EXAMPLE 31

Fibronectin in Foam Wound Dressing

10 grams soy lecithin and 50 mg of fibronectin was impregnated into foam as in Example 27. SDMC's were consistent with the size structure of other SDMCs, having a mean size of 190 nm.

EXAMPLE 32

Stability of Passenger Molecule Activity Over Time

Antibacterial activity of the 2.5% PCMX containing SDMCs after thirty days room temperature storage and 1 hour at 70.degree. C. was tested by spotting 20 ul formed solution in the center of a lawn of *E. coli* strain Y1089. (The lawn was created by growing Y1089 at 30.degree. C. in Luri Broth [LB] broth until an optical density of 0.1 at 600 nm was achieved). Twenty ul of the *E. coli* suspension was spotted in the center of a LB plate and spread with a sterile inoculating loop. Plates to be tested were held for 18 hours at 30.degree. C. until the antibacterial effects could be seen and measured in the confluent lawn of *E. coli*.

It was shown that the small amount of residual solvent and other components used to entrap (encapsulate) PCMX had no inhibitory effect on microbial growth (no zone of growth inhibition was seen).

A 3% solution of non-encapsulated PCMX was spotted in a like manner and was found to create a 8 mm zone of growth inhibition compared to SDMC. Encapsulated PCMX created a 25 mm zone. No significant reduction in the ability of encapsulated PCMX to inhibit bacterial growth was noted when procedures using 30 day old or heat treated SDMCs (30 day old SDMCs heated at 70.degree. C. for one hour) were compared.

EXAMPLE 33

Comparison of PCMX SDMC Activity to a Commercial Preparation of PCMX

The antibacterial activity of SDMCs containing PCMX (2.5%) as a passenger molecule was compared against a commercial preparation of PCMX (Ultradox (Dexide Inc.) (3%)). Both solutions were diluted 1:2 v/v in LB broth using standard bacteriostatic and bacteriocidal assay techniques (1 ml total volume). After dilution of the two antimicrobial preparations, 0.1 ml of a 0.1 O.D. solution of *E. coli* Y1089 was added to each dilution tube. All tubes were incubated at 30.degree. C. for 18 hours. After the incubation, each dilution was tested for microbial growth by removing a small amount of broth using a sterile inoculating loop and streaking on a LB plate. After incubating the plates for 18 hours at 30.degree. growth inhibition at each dilution was determined by examining the plates for growth of Y1089. Bacterial growth inhibition by Ultradox (3%) stopped a dilution of 1:64 v/v. The 2.5% PCMX SDMC preparation inhibited growth up to a dilution of 1:1024. Therefore, the preparation has 16-32X better bacteriocidal activity than the Ultradox 3% solution.

EXAMPLE 34

A mouthwash was formulated employing 2.5% PCMX. Antibacterial activity of the mouthwash without PCMX was tested and no inhibition of *E. coli* was found using the standard plate inhibition assay, even though the mouthwash contained 70% ethanol. A 2.0% solution of PCMX in the mouthwash was prepared and upon testing found to achieve a zone of 19 mm compared to a 25 mm zone which was created by only a 0.2% solution of a SDMC encapsulated preparation.

EXAMPLE 35

A new encapsulated 5% PCMX suspension was tested using the *E. coli* plate inhibition assay. The control plate showed no zone of bacterial inhibition compared to the free PCMX that had a zone approximately 20 mm. The 5% free suspension did not achieve a complete inhibition of bacteria within the 20 mm zone. The SDMC preparation in contrast, showed complete inhibition of bacterial growth with a 35 mm zone due to the solubilization of the agent and even distribution over the surface.

EXAMPLE 36

SDMC Size Distribution, Time and Heat Stability

The size distribution of solvent dilution microcarriers containing 2.5% PCMX was tested using a Coulter NM4A multi-angle sub micron particle analyzer as previous described. Batch A was prepared on the same day as Batch B and as can be seen in Table I, the size of the SDMCs is fairly homogenous and stable over time and after heating at 70.degree. C. for 1 hour.

TABLE I

SDMC	Size (Day 1)	Size (Day 30)	Size Day 30 after Heating at 70.degree. C. for 1 hour
Batch A	130-230 nm (mean 180)	105-190 nm (mean 169)	100-140 nm (mean 123)
Batch B	180-300 nm (mean 227)	NT	NT

NT = Not Tested

EXAMPLE 37

SDMCs in a Vaginal Sponge Contraceptive

SDMCs would be prepared as described in Example 2, except that estrogen or estrogen-like compounds would be utilized as the passenger molecule. In the alternative, a spermicidal agent such as nonoxynol-9 could be employed as the passenger molecule.

EXAMPLE 38

Sustained Release of Tobramycin SDMC From Foam Wound Dressing

To a solution of 75 ml of dichloromethane was added 10 grams of soy lecithin and 200 mg of tobramycin sulfate in 5 ml of water. The solution was mixed by shaking and 4 ml were applied onto each side of the 1".times.1" foam bandage material and allowed to air dry. Four ml of a solution comprising 20 mg of tobramycin sulfate in 8 ml of water was applied onto each side of 10 additional foam bandage material pieces (1".times.1") and allowed to air dry. The foam bandage pieces were placed onto a grid support, wetted with 5 ml of water and 2 ml of eluent was collected which flowed through the foam dressing and into the underlying receptacle. The foam dressings were wetted subsequent times and 2 ml of eluent was collected. The antimicrobial activity of eluent was determined using standard antimicrobial inhibition assays using E. coli strain Y-1089. The results as shown in Table II.

TABLE II

Release of Tobramycin From Foam Dressing		
Collection Point	Aqueous Tobramycin Concentration	SDMC Entrapped Tobramycin mg per 2 ml
1	19.0	0.50
2	0.50	0.50
3	0.10	0.50
4	0.10	0.55
5	less than	0.10
6	N.D.*	0.45
7	N.D.	0.45
8	N.D.	0.50
9	N.D.	0.50
10	N.D.	0.50
11	N.D.	0.45

12	N.D.	0.45
13	N.D.	0.50
14	N.D.	0.45
15	N.D.	0.50
16	N.D.	0.50
17	N.D.	0.55
18	N.D.	0.50

*N.D. = Not Detected

EXAMPLE 39

Sustained Release of SDMC from Foam Wound Dressing

To a solution of 75 ml of dichloromethane was added 10 grams of soy lecithin and tracer amounts of ¹⁴C-Dipalmitoylphosphatidylcholine (DPPC) and 5 ml of water. The solution was mixed by shaking and 4 ml were applied onto each side of a 1".times.1" foam bandage material and allowed to air dry. The foam bandages were placed onto a grid support, wetted with 5 ml of water and 2 ml of eluent was collected. Eluent samples were assayed by liquid scintillation counting techniques. The results demonstrated that uniform amounts (between 1-3%) of SDMCs were collected after each application of fluid. Results indicated that over 25 applications of fluid were required to exhaust the foam bandage of SDMCs.

EXAMPLE 40

Entrapment of Tobramycin in SDMC as It Elutes from Foam Wound Dressing

To a solution of 75 ml of dichloromethane was added 10 grams of soy lecithin (with tracer amounts of ³²P-phosphatidylcholine) and 200 mg of tobramycin sulfate in 5 ml of water. The solution was mixed by shaking and 4 ml were applied onto each side of 1".times.1" foam bandage material and allowed to air dry. The bandage materials were placed onto a grid support as in Example 1 and wetted with 5 ml of water. The eluents for the first nine wettings were discarded. After ten repeated wettings the eluent was collected and passed over a Sephadex G-150 column. The material which was excluded by the column and included by the column bed was assayed for tobramycin using standard antimicrobial assays with E. Coli strain Y-1089. The material which was excluded was subjected to turbidity measurements and laser light scattering analysis. The sizes of the SDMCs were consistent with those previously observed between 0.15 μ m and 0.3 μ m and when assayed for tobramycin demonstrated that the antibiotic was indeed entrapped within the SDMCs which eluted from the foam bandage material. The included volume contained a small amount (less than 10% of the total tobramycin) which had either not been entrapped in SDMCs or had leaked out of SDMCs during transit from foam dressing or over the Sephadex column.

EXAMPLE 41

Stability of SDMC Encapsulated Materials As They Leave Foam Bandage Material

To a solution of 50 ml of dichloromethane was added 10 grams of soy lecithin, 6.9.times.10.⁷ I.U. of tetrachlorodecaoxid ("TCDO") and 5 ml of water, 50 ml of additional dichloromethane was added and the resulting solution was mixed by shaking. Four ml of solution were applied onto each side of 1".times.1" foam bandage material and allowed to air dry. Eight ml (4 ml onto each side) of a solution containing 5.0.times.10.⁶ I.U. of TCDO was applied onto an additional 1".times.1" foam bandage material. The bandage materials were placed onto a grid support, wetted with 5 ml of water and 2 ml aliquots were collected from both the SDMC entrapped TCDO sponges and the sponges impregnated with TCDO alone. The aliquots were analyzed for antimicrobial activity immediately and stored at room temperature. At subsequent times (for 48 hours) aliquots were again assayed for TCDO activity. The results of the percentage of initial activity retained in each aliquot is shown in Table III.

TABLE III

Stability of SDMC Entrapped TCDO Eluent
From Foam Wound Bandage
Hours After Percentage of Initial Antimicrobial Activity

Elution From Bandage	TCDO	SDMC Entrapped TCDO
0	100	100
2	40	85
4	20	65
8	less than 1	60
24	N.D.*	55
36	N.D.	50
48	N.D.	50

*N.D. = Not Detected

EXAMPLE 42

Stability of SDMC Entrapped Material in Wet Bandage Environment

Foam bandage materials were prepared as in Example 4 (with either TCDO or SDMC entrapped TCDO). The foam bandage materials were placed onto a support grid and wet with 5 ml of water. Two ml eluent was collected at 4 hours and at 12-hour intervals for 4 days. The percentage of initial antimicrobial activity at each collection time was determined using standard antimicrobial assays. The results are shown in Table IV.

TABLE IV

Stability of SDMC Entrapped TCDO In Wet Foam Wound Bandage Hours After		
Wetting of Bandage	Percentage of 4 Hour Antimicrobial Activity TCDO	SDMC Entrapped TCDO
4	100	100
12	less than 1	85
24	N.D.*	85
36	N.D.	85
48	N.D.	80
60	N.D.	80
72	N.D.	80
84	N.D.	80
96	N.D.	80

*N.D. = Not Detected

EXAMPLE 43

Stability of SDMCs to Osmotic Stress

To a solution of 25 ml of dichloromethane was added 5 grams of soy lecithin and 5 ml of water. Twenty-five ml of additional dichloromethane was added and the resulting solution was mixed by shaking. Four ml of solution were applied onto each side of 1".times.1" foam bandage material and allowed to air dry. The foam bandages were placed onto a grid support, wetted with 5 ml of water and 2 ml aliquots of eluent were collected. To aliquots of eluent containing empty SDMCs were added increasing concentrations of sucrose or water, thus placing the SDMCs under increasing osmotic stress. Changes in SDMCs were monitored using laser light scattering analysis. Results are shown in Table V.

TABLE V

Resistance of SDMCs to Increased Osmotic Stress

Micro Osmoles	Percentage	Size of SDMC
Osmotic	Sucrose	NM
Pressure		

539	0	125 .+- . 10
1,100	5	125 .+- . 20
2,156	10	150 .+- . 30
4,312	20	150 .+- . 30
6,624	30	150 .+- . 30
17,248	40	150 .+- . 30

EXAMPLE 44

Stability to SDMCs to Detergent Stress

SDMCs were prepared as in Example 6. To the eluent containing empty SDMCs were added either water or increasing amounts of Tween 20 (a commercial detergent). The effects on size of SDMCs was monitored using laser light scattering analysis. Results are shown in Table VI.

TABLE VI

Resistance of SDMCs to Increased Detergent Concentration	
Percentage Volume/Volume	Size of SDMCs
Tween 20	(NM)
0	190
0.15	190
0.3	190
0.75	190
1.2	205

EXAMPLE 45

Thermal Stability of Dry Tobramycin SDMC Forming Foam Wound Dressings

To a solution of 50 ml of dichloromethane was added 10 grams of soy lecithin and 200 mg of tobramycin sulfate in 5 ml of water. Fifty ml of additional dichloromethane was added and the solution was mixed by shaking. Eight ml of solution (4 ml per side) was added to a 1".times.1" foam wound bandage material which was then allowed to air dry. The materials were analyzed for residual dichloromethane by high pressure liquid chromatography and found to contain no detectable residual dichloromethane. The 1".times.1" foam wound materials were packaged individually in heat sealed bags and stored at room temperature for two months. At the end of two months the packages were opened and a representative first sample of SDMC-forming foam wound bandages were removed, placed onto grid supports, and wetted with 5 ml of water. Two ml of eluent containing SDMC-entrapped tobramycin were collected from the first sample. A second representative sample of SDMC-forming foam wound bandages were stressed by heating to +70.degree. C. for three days. A third representative sample of SDMC-forming foam wound bandages were stressed by freezing at -70.degree. C. for three days, then allowed to equilibrate to room temperature. These stressed foam wound dressings were then placed onto grid supports, wetted with 5 ml of water, and 2 ml of eluent containing SDMC-entrapped tobramycin was collected. All samples were subjected to analysis for tobramycin entrapped as explained in Example 3. The results are shown in Table VII.

TABLE VII

Effect of Thermal Stress of Tobramycin Entrapped
in SDMC Forming Foam Wound Dressing

Concentration of Tobramycin
Temperature Entrapped in SDMC (mg per ml)

Room Temperature	0.5 mg/ml
+70.degree. C.	0.5 mg/ml
-70.degree. C.	0.5 mg/ml

EXAMPLE 46

Thermal Stability of Dry Silver Sulfadiazine SDMC-Forming Foam Wound Dressing

To a solution of 10% weight per volume soy lecithin in ethanol (192 ml) was added 418 grams of methyl paraben and mixed by shaking. To the resulting solution was added 4.8 grams of silver sulfadiazine and mixed by shaking. One-hundred and twenty ml of Tween 20 was added to the solution which was then mixed by shaking. Dichloromethane (720 ml) was then added to the solution and mixed by shaking. Sixteen ml of this solution was placed on each side of a 4".times.4" foam wound bandage material. This was allowed to air dry. The 4".times.4" foam wound material was cut into 1".times.1" square pieces. One representative sample of 1".times.1" pieces was placed onto a grid support and wetted with 5 ml of water. Two ml of eluent containing SDMC silver sulfadiazine was then collected. A second representative sample of the 1".times.1" pieces of foam wound material was stressed by heating to +70.degree. C. for three days. A third representative sample of the 1".times.1" piece was stressed by freezing at -85.degree. C. for three days, then allowing equilibration to room temperature. These stressed foam wound dressings were then placed onto grid and treated as above. Eluent was collected-after each of the ten applications of 2 ml of water to each 1".times.1" square test piece. All samples were subjected to analysis for silver sulfadiazine antimicrobial activity using standard microbiological techniques. The results are shown in Table VIII.

TABLE VIII

Effect of Thermal Stress of Silver Sulfadiazine
Entrapped in SDMC Forming Wound Dressing
Concentration of Silver Sulfadiazine
Entrapped in SDMC Collection Point
Temperature First Elution Tenth Elution

Room Temperature	21	20
-85.degree. C.	20	21
+70.degree. C.	20	21

EXAMPLE 47

Sustained-Release of SDMC Entrapped Tobramycin from Wet Foam Wound Material Over Time

Foam wound materials containing tobramycin sulfate were prepared as shown in Example 1. Once the materials were placed onto grid supports they were maintained at room temperature and covered with a foil wrap. Every 12 hours for nine days, 5 ml of water was applied onto the foam bandage materials and eluent was collected. Two ml samples containing SDMC-entrapped tobramycin were assayed for tobramycin antibiotic activity using standard antimicrobial assays. The results are shown in Table IX.

TABLE IX

Sustained Release of Tobramycin from SDMC Forming Wound Dressing	
Time (Days)	SDMC Tobramycin Concentration (.mu.g/ml)
0	400
0.5	410
1.0	415
1.5	415
2.0	415
2.5	405
3.0	405
3.5	390
4.0	400
4.5	405
5.0	405
5.5	410
6.0	405
6.5	410
7.0	410
7.5	410
8.0	415
8.5	405
9.0	410

EXAMPLE 48**Sustained Release of SDMC Entrapped Gentamicin From Wet Foam Wound Material Over Time**

Foam wound materials containing gentamicin sulfate were prepared as shown in Example 1. Once materials were prepared they were placed onto grid supports and maintained at room temperature. All were covered with a foil wrap. Every 12 hours for nine days, 5 ml of water was applied onto the foam bandage materials and eluent was collected. Two ml samples containing SDMC entrapped gentamicin were assayed for gentamicin antibiotic activity using standard antimicrobial assays. The results are shown in Table X.

TABLE X

Sustained Release of Gentamicin From Wet SDMC Forming Wound Dressing	
Time (Days)	SDMC Entrapped Gentamicin Concentration (.mu.g/ml)
0	200
0.5	200
1.0	205
1.5	205
2.0	205
2.5	205
3.0	200
3.5	175
4.0	190
4.5	200
5.0	200
5.5	200
6.0	200

6.5	205
7.0	195
7.5	200
8.0	200
8.5	200
9.0	205

EXAMPLE 49

Preparation and Sterilization of Polymyxin B SDMCs in Foam Wound Dressing

To a solution of 125 ml of dichloromethane was added 500,000 units (10 ml of aqueous solution) of polymyxin B. The resulting solution was mixed by shaking. To the solution was added 20 ml of soy lecithin (Alcolec x-tra A) and the resulting solution was mixed by shaking. Four ml of above solution was added to each side of a 1".times.1" foam wound material which was allowed to air dry. The materials were placed into heat sealed pouches and sterilized by use of cobalt irradiation (3.2 megarads). The resulting materials were assayed by placing them onto grid supports, wetting with 5 ml [water] and collecting 2 ml of eluent containing SDMC entrapped polymyxin B. The eluent contained fully active entrapped antibiotic after irradiation as determined by use of standard antimicrobial-assay techniques.

EXAMPLE 50

Preparation and Sterilization of Kanamycin SDMCs in Foam Wound Dressing

To a solution of 125 ml of dichloromethane was added 1 gram of kanamycin sulfate in 10 ml of aqueous solution. To the resulting solution was added 20 ml of soy lecithin (Alcolec S). The solution was mixed by shaking. Four ml of the solution was applied to each side of a 1".times.1" foam wound dressing, which was then allowed to dry. These materials were placed in foil heat sealed bags and subjected to sterilization by use of cobalt irradiation (3.2 megarads). The materials were assayed for antibiotic activity as described in Example 12. The eluent contained fully active entrapped antibiotic after irradiation by using standard antimicrobial assay techniques.

EXAMPLE 51

Sustained Release of SDMC Entrapped Silver Sulfadiazine From Wet Foam Wound Materials Over Time

Foam wound materials were prepared as described in Example 9. Once the materials were placed onto grid supports, they were maintained at room temperature and covered with a foil wrap. Every 12 hours for seven days, 5 ml of water was applied onto the foam bandage materials and eluent was collected. Two ml samples containing SDMC entrapped silver sulfadiazine were assayed for silver sulfadiazine antimicrobial activity using standard microbiological techniques. The results are shown in Table XI.

TABLE XI

Sustained Release of Silver Sulfadiazine From Wet SDMC Forming Foam Wound Dressing	
SDMC Entrapped Silver Sulfadiazine	
Time (Days)	Concentration (.mu.g/ml)
0	18
0.5	20
1.0	20
1.5	19
2.0	20
2.5	20
3.0	21
3.5	20

4.0	19
4.5	20
5.0	21
5.5	20
6.0	21
6.5	20
7.0	20

EXAMPLE 52

Preparation and Sterilization of Cefoxitin Entrapped in SDMCs

To a solution of 15 ml of 10% (weight per volume) NaCl in water was added 4 grams of cefoxitin. The mixture was shaken and resulting SDMCs entrapping cefoxitin were sterilized using Cobalt irradiation (2.5 megarads). The SDMCs were assayed for cefoxitin antimicrobial activity using standard microbiological techniques and found to both entrap the cefoxitin and preserve its activity both during one week storage at 4.degree. C. and through irradiation sterilization.

EXAMPLE 53

Preparation and Sterilization of Amakacin Entrapped in SDMCs

To a solution of 15 ml of 10% (weight per volume) soy lecithin (Alcolec LKE granules) was added 85 ml of 0.95% (weight per volume) NaCl in water containing 2 grams of amakacin sulfate. The mixture was shaken and resulting SDMCs entrapping amakacin were sterilized using cobalt irradiation (2.5 megarads). The SDMCs were assayed for amakacin antimicrobial activity using standard microbiological methodology and found to both entrap amakacin and preserve the activity of the antibiotic both during storage at 4.degree. C. and through irradiation sterilization.

EXAMPLE 54

Preparation of Kanamycin Entrapped SDMCs

To a 40 ml solution of 0.05 potassium phosphate (pH 7.0) was added 1 gram of tobramycin sulfate. Three ml of a 10% (weight per volume) soy lecithin in ethanol solution was added to tobramycin containing solution. The resulting SDMC containing mixture was swirled to entrap tobramycin with SDMCs. To adjust volume 57 ml of 0.05 M potassium phosphate was added and resulting suspension was passed through a 0.45 μ m filter into a sterile vial.

EXAMPLE 55

Preparation of a Stable Cyclosporin Admixture

To 2 ml of a 10% (weight per volume) soy lecithin in ethanol solution was added 3 mg of cyclosporin. The solution was mixed by swirling. To form SDMC 50 μ l aliquot of above solution was mixed with 450 μ l of water. The resulting SDMCs containing cyclosporin were analyzed using laser light scattering and found to be approximately 200 μ m in diameter. The admixture was stored at room temperature for one year and subjected to testing as above. The results found no change in size (200 μ m diameter) and no precipitation of cyclosporin in the admixtures during storage for one year.

EXAMPLE 56

Use of Novel Wound Dressing Package Containing Gentamicin Sulfate for Treatment of Soft Tissue Infections in Rats

A model of soft tissue infection was established in Sprague Dawley rats. Rats were anesthetized and a 3.times.3 cm square of skin was excised from the back exposing paraspinus muscles. One-half ml of a suspension containing 1.times.10.sup.8 cfu/ml of Pseudomonas aeruginosa was injected into the superficial fascia of each paraspinus muscle

for a total volume of 1 ml. Wounds were covered with a non-adherent dressing material (N-terface.RTM. interpositioned surfacing material, available from Winfield Laboratories, Richardson, Tex. 75083) and either a foam wound material or a predried foam wound dressing prepared by impregnation of a SDMC forming solution consisting of 0.5 grams of soy lecithin and 2 mg gentamicin sulfate applied onto 1".times.1" foam material (prepared as described in Example 1). In all cases bandage materials were moistened with 5 ml of either sterile water for 40 rats, 5 ml of sterile water for rats receiving SDMC gentamicin (40 rats) or 5 ml of sterile water containing 330 .mu.g of gentamicin sulfate (40 rats). Animals were redampened every 12 hours as above for a duration of three days. During the three-day study at 24-hour intervals, groups of ten rats were sacrificed and the paraspinus muscle harvested obtaining between 1.5 and 3 grams of tissue. Colony forming units (CFU) of *P. aeruginosa* [per] 1 gram of muscle tissue was determined using standard microbiological techniques. The results for each treatment group are shown in Table XII.

TABLE XII

Treatment of Soft Tissue Infections with Gentamicin				
Hours after Treatment (percent of time 0 bacteria per gram of tissue)				
Treatment Groups	0	24	48	72
Untreated Controls				
100		>100	>100	>100
Aqueous Gentamicin				
100		>100	>100	>100
SDMC Gentamicin				
100		45	2	<0.01

EXAMPLE 57

Use of Novel Wound Dressing Package Containing Silver Sulfadiazine for Treatment of Soft Tissue Infection in the Rat

A model of soft tissue infection was established in Sprague Dawley rats as described in Example 17. All animals were treated as described in Example 17 with the following exceptions. Treatment groups consisted of 1) animal receiving foam wound dressing (wet with sterile water), 40 animals, 2) animals receiving 3 grams of 1% silver sulfadiazine (30 mg) (Silvadene.RTM. Cream) applied into the wound, scrubbed away and reapplied twice daily for three days (40 animals) and 3) animals receiving a pre-dried foam wound material impregnated with SDMC forming solution consisting of 30 mg of silver sulfadiazine and 0.5 grams of soy lecithin (prepared as described in Example 9). The results for each treatment group are shown in Table XIII.

TABLE XIII

Treatment of Soft Tissue Infections With Silver Sulfadiazine				
Hours after Treatment (percent of time 0 bacteria per gram of tissue)				
Treatment Groups	0	24	48	72
Untreated Controls				
100		>100	>100	>100
Silvadene Cream				
100		68	40	22
SDMC Silver Sulfadiazine				
100		40	12	<0.01

EXAMPLE 58

Use of SDMC Entrapped Cefoxitin to Treat Fatal Bacteria Septicemia Resulting From Mixed Bacterial Peritonitis

A model for fatal septicemia resulting from mixed bacterial infections were established in Sprague Dawley rats. Rats were anesthetized and a midline incision made into abdomen. The peritoneal cavity was irritated with a barium solution and 1 gram of human fecal material was implanted into the peritoneal cavity. Animals were surgically closed. Blood samples were removed from caudal vein at zero, four hours and subsequent 24-hour intervals for the duration of a four-day study. Infections were established in a group of 40 animals. In a group of ten animals, no treatment was initiated. In a group of ten animals, cefoxitin 1.5 mg/kg was administered i.m. at the time of surgical closure. In a group of ten animals, cefoxitin 1.5 mg/kg was administered into the peritoneal cavity at the time of surgical closure. In a group of ten animals, SDMC-entrapped cefoxitin 1.5 mg/kg was administered at the time of surgical closure. In a group of ten animals, empty SDMCs were administered i.p. at the time of surgical closure. The results of this study are shown in Table XIV.

TABLE XIV

Treatment of Fatal Septicemia Resulting From Mixed Bacterial Peritonitis				
Bacteria (cfw/ml) In Blood Per Ml				
Hours After Treatment				
Treatment Groups	0	4	24	48
				72
Controls	0	1	.times.	10.sup.4
			1	.times. 10.sup.7
				>1 .times. 10.sup.7
				>1 .times. 10.sup.7
Cefoxitin (i.m.)	0	1	.times.	10.sup.3
			1	.times. 10.sup.4
				1 .times. 10.sup.4
				1 .times. 10.sup.4
Cefoxitin (i.p.)	0	1	.times.	10.sup.3
			1	.times. 10.sup.4
				1 .times. 10.sup.4
				1 .times. 10.sup.4
SDMC cefoxitin (i.p.)	0	>1	.times.	10.sup.1
			>1	.times. 10.sup.1
				>1 .times. 10.sup.1
				>1 .times. 10.sup.1
Empty SDMCs	0	1	.times.	10.sup.3
			1	.times. 10.sup.6
				1 .times. 10.sup.6
				1 .times. 10.sup.6

EXAMPLE 59

Prevention of Fata Septicemia Resulting from Mixed Bacterial Peritonitis By Use of SDMC-Entrapped Cefoxitin

A model for fatal septicemia resulting from mixed bacterial infections were established in Sprague Dawley rats as described in Example 58. The mortality of each of the treatment groups is reported in Table XV.

TABLE XV

Mortality Due To Fatal Septicemia Results From Mixed Bacterial Peritonitis		
Mortality In Animals Following Infection		
Treatment Groups	4 hours	72 hours
Control	0	9/10 (90%)
Cefoxitin im/ip	0	4/10 (40%)
SDMC Cefoxitin	0	0 (0%)

EXAMPLE 60

Reduction of Initial Serum Blood Levels of SDMC-Entrapped Tobramycin Sulfate Following Intraperitoneal Administration

To evaluate the effect of SDMC-entrapped tobramycin on serum blood levels, SDMCs were prepared as follows: to a 40 ml solution of 0.95% saline containing 1 gram of tobramycin sulfate was added 3 ml of a 10% (weight per volume) soy lecithin (Alcolec LKE granules). The SDMC-entrapping tobramycins were formed and suspension mixed by swirling. An additional 60 ml of 0.95% saline was added and suspension swirled. Groups of five animals received either 1 ml of saline i.p., 1 ml of aqueous tobramycin sulfate (10 mg) i.p. or SDMC-entrapped tobramycin (10 mg). Animals were bled by caudal vein puncture prior to treatment and at four and twenty-four hours after treatment. Seven levels of tobramycin were determined using standard assay techniques. The results of experiments are shown in Table XVI.

TABLE XVI

Serum Concentrations of Tobramycin Following I.P. Administration			
Serum Concentrations (mg/ml) of Tobramycin			
Experimental	Time of Administration		
Groups	0	4	24
Control	0	0	0
Tobramycin	0	4.5 \pm 0.7	1.3 \pm 0.4
SDMC	0	1.1 \pm 0.1	1.4 \pm 0.1
Tobramycin			

EXAMPLE 61

Stabilization of Methoprene to Ultraviolet Light Degradation by SDMC Encapsulation

Methoprene, 90% technical grade, was encapsulated into SDMC in the following manner. To 10 ml of a, soy ecithin (Alcolec X-tra.sup.A) was added 1 ml of Tween 20 and 1 ml of methoprene. To above mixture was added 5 ml of ethanol and swirled. To the resulting mixture 85 ml of water was added and mixed by swirling. To evaluate

ultraviolet light protection by encapsulation of methoprene in SDMCs, standardized flea egg hatching studies were done on environmental surfaces (carpet, 1 ft.times.1 ft. square). Carpet samples received either no treatment, 1% methoprene applied in volatile propellant or methoprene entrapped in SDMCs as prepared above. Flea eggs were applied to the carpet samples and the percent of initial methoprene activity was determined as a function of the percentage of flea eggs which hatched. All carpet samples were exposed to ultraviolet light daily during the study period. The carpet samples were reinfected for three subsequent months. The percentage of methoprene activities for each of the experimental groups are shown in Table XVII.

TABLE XVII

Experimental Groups	Protection of Methoprene From Ultraviolet Light Degradation Percentage of Initial Pesticide Activity			
	Time After Application	0	1	2
Months	0	1	2	3
Control	0	0	0	0
Methoprene	100	0	0	0
SDMC Methoprene	100	92	89	90

EXAMPLE 62

Reduction of Odor By Encapsulation of Propetamphos by SDMCs

Propetamphos was encapsulated into SDMCs in the following manner. To a 10 ml of a soy lecithin (10% weight per volume in ethanol) was added 1 ml of Tween 20 and 1 ml of propetamphos. To the above mixture was added 90 ml of water and mixed by swirling. A noticeable reduction in offensive odor was observed when compared to equal amounts of propetamphos in ethanol above.

EXAMPLE 63

SDMC Containing Retinoic Acid

A sample of 100 mg of soy lecithin was solubilized at room temperature with 30 mg of retinoic acid in ethanol. One ml of water was added to the solution which resulted in a turbid (cloudy) suspension. Three ml of absolute ethanol was added to the suspension to yield an optically clear solution. SDMCs were formed by dilution of 1 ml of final solution into 10 ml of aqueous solution.



Encapsulation in the food industry: a review

Bernard F. Gibbs,^{1,2} Selim Kermasha,¹ Inteaz Alli¹ and Catherine N. Mulligan^{2,3}

¹*Department of Food Science and Agricultural Chemistry, Macdonald Campus, McGill University, 21, 111 Lakeshore, Ste-Anne-de-Bellevue, Quebec H9X 3V9, ²Bivan Consultants, Food Biotechnology Division, Suite 1415, 6550 Sherbrooke St., Montreal, Quebec H4B 1N6, Canada and ³Concordia University, 1455 de Maisonneuve Blvd. W, Montreal, Quebec H3G 1M8, Canada*

Encapsulation involves the incorporation of food ingredients, enzymes, cells or other materials in small capsules. Applications for this technique have increased in the food industry since the encapsulated materials can be protected from moisture, heat or other extreme conditions, thus enhancing their stability and maintaining viability. Encapsulation in foods is also utilized to mask odours or tastes. Various techniques are employed to form the capsules, including spray drying, spray chilling or spray cooling, extrusion coating, fluidized bed coating, liposome entrapment, coacervation, inclusion complexation, centrifugal extrusion and rotational suspension separation. Each of these techniques is discussed in this review. A wide variety of foods is encapsulated – flavouring agents, acids, bases, artificial sweeteners, colourants, preservatives, leavening agents, antioxidants, agents with undesirable flavours, odours and nutrients, among others. The use of encapsulation for sweeteners such as aspartame and flavours in chewing gum is well known. Fats, starches, dextrans, alginates, protein and lipid materials can be employed as encapsulating materials. Various methods exist to release the ingredients from the capsules. Release can be site-specific, stage-specific or signalled by changes in pH, temperature, irradiation or osmotic shock. In the food industry, the most common method is by solvent-activated release. The addition of water to dry beverages or cake mixes is an example. Liposomes have been applied in cheese-making, and its use in the preparation of food emulsions such as spreads, margarine and mayonnaise is a developing area. Most recent developments include the encapsulation of foods in the areas of controlled release, carrier materials, preparation methods and sweetener immobilization. New markets are being developed and current research is underway to reduce the high production costs and lack of food-grade materials.

Introduction

Approximately 30 years ago, encapsulation processes were developed. It involves the coating or entrapment of a pure material or a mixture into another material. The coated or entrapped material is usually a liquid but can be a solid or gas. This material is also known as the core material, actives, fill, internal

phase or payload. The coating material can also be called the capsule, wall material, membrane, carrier or shell. The purpose of encapsulation is to protect its contents from the environment which can be destructive while allowing small molecules to pass in and out of the membrane. Natural examples

Correspondence to: Bernard F. Gibbs.

include birds' egg shells, plant seeds, bacterial spores, skin and seashells.

Early versions of microcapsules were impermeable and were broken apart, most often by mechanical means, for the inner ingredients to become active. Examples included controlled release of flavours and aromas, perfumes, drugs, detoxicants, fertilizers and precursors in textiles and printing (Seiss & Divies, 1981). Enzymes, plant, animal or microbial cells could be encapsulated to allow substrates to enter the membrane and products to leave. This concept was instrumental in the development of artificial kidneys since detoxifying enzymes could be placed in semipermeable membranes (Chang, 1978) and perform the function of the kidney. Nylon membranes have been used by Desoize (1986) to encapsulate and cross-link enzymes such as casein and pepsin. Examples of enzyme encapsulation include juice clarification with pectin esterase, sucrose inversion by invertase and milk coagulation with rennet (Lee, 1996).

An important bacteria used in the industry, lactic acid bacteria, was first immobilized in 1975 on Berl saddles and *Lactobacillus lactis* was encapsulated in alginate gel beads years later (Linko, 1985). Seiss and Divies (1981) suggested that immobilized lactic acid bacteria could be used to continuously produce yoghurt. However, the alginate beads of *L. lactis* susp. *cremoris* leaked large quantities of cells. Other membranes such as poly-L-lysine, nylon and polyethyleneimine to coat alginate beads have also recently been examined (Larisch, 1990) but did not show any improvement in lactic acid production as compared to free cells.

Encapsulation involves the incorporation of various ingredients within a capsule of approximately 5 to 300 micron in diameter (Lee, 1996). The capsule can be made of sugars, gums, proteins, natural and modified polysaccharides, lipids and synthetic polymers. The advantages of encapsulation include improved flow properties and easier handling since they are solid instead of liquid. Stability of the encapsulated material can be improved due to protection from moisture or heat.

Encapsulation can be of many different forms such as a simple membrane coating, a wall or membrane of spherical or irregular shaped, a multiwall structure with walls of the same or varying compositions or numerous cores within

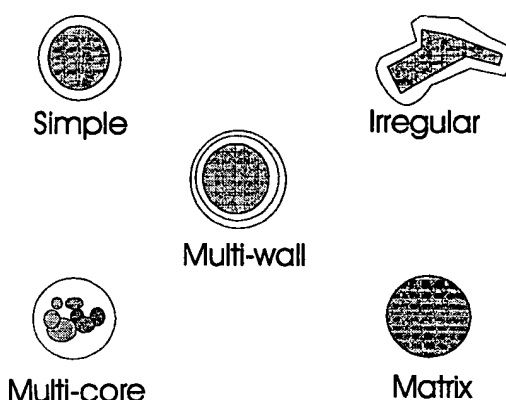


Figure 1. Various forms of capsules.

the same walled structure as shown in Figure 1. Currently, almost any material can be encapsulated for the purpose of isolation, purification or slow release.

For many years, this technique has been used in the pharmaceutical industry for time-release, enhanced stability of formulations and flavour masking. Prescription drugs, over-the-counter drugs, vitamins and minerals have been encapsulated. Therefore, these applications, in addition to many others, would be useful in the food industry.

Applications have been slower in increasing since the technique was thought to be too expensive and highly specific. However, since production volumes have increased and more cost-effective preparation techniques and materials have been developed, the number of encapsulated food products has significantly increased. Microcapsules can improve nutrition since the extensive storage of many products can result in the loss of nutritional value by enabling the addition of oxidation-sensitive vitamins, minerals and proteins to various products.

Manufacturing techniques

Various techniques are used for encapsulation (Dziezak, 1988). In general, three steps are involved: formation of the wall around the material, ensuring that leakage does not occur, and ensuring that undesired materials are kept out. These encapsulation techniques include spray drying, spray chilling or spray cooling,

extrusion coating, fluidized bed coating, liposome entrapment, coacervation, inclusion complexation, centrifugal extrusion and rotational suspension separation. Each of these methods will be discussed in the following sections.

Spray drying

Since spray drying is an economical, effective method for protecting materials and specialized equipment is not required, it is most widely employed, particularly for flavours. It is also used for dehydration of materials such as powdered milk. For encapsulation purposes, modified starch, maltodextrin, gum or others are hydrated to be used as the carrier or wall material. The material for encapsulation is homogenized with the carrier material usually at a ratio of 1 : 4. The mixture is then fed into a spray dryer and atomized with a nozzle or spinning wheel. Water is evaporated by the hot air contacting the atomized material. The capsules are then collected after they fall to the bottom of the drier.

Recent developments have been in the use of new carrier materials and a newly designed spray dryer. Colloides Naturels (Thevenet, 1995) and TIC Gums (Reineccius *et al.*, 1995) have developed new combinations of gum arabic starches to increase the retention of volatiles and shelf-life of the microcapsules. In particular, Risch and Reineccius (1988) enhanced the retention of orange oil and decreased oxidation by using gum arabic. Bhandari *et al.* (1992) showed that a new type of dryer called the Leafish spray dryer, which uses a high air velocity with a temperature of 300 to 400°C, was effective for encapsulating citral and linalyl acetate without degradation. A disadvantage is that a separate agglomeration step is required to prevent separation or to render the obtained powder soluble. A chief advantage is that this technique can be used for heat-labile materials.

Spray chilling or spray cooling

In spray chilling, the material to be encapsulated is mixed with the carrier and atomized by cooled or chilled air as opposed to heated air as in spray drying (Risch, 1995). The outer material is usually vegetable oil in the case of spray cooling (45 to 122°C) or a hydrogenated or fractionated vegetable oil in the case of spray chilling (32 to 42°C). The disadvantage of the

latter method is that special handling and storage conditions could be required (Taylor, 1983). Spray chilling is usually used for ferrous sulfate, vitamin, mineral or acidulent encapsulation. Frozen liquids, heat-sensitive materials and those not soluble in the usual solvents can be encapsulated in this manner. These materials are then released as the wall material is melted. Applications of spray chilling can include: dry soup mixes, foods with high fat contents and bakery products (Blenford, 1986).

Extrusion

Extrusion was first patented in 1957 (Swisher, 1957) and further developed by the same group. At this time, citrus oils were dispersed in corn syrup solids and glycerine at 125°C as heated by steam, poured into a chamber pressurized by nitrogen and extruded into a dehydrating liquid such as isopropyl alcohol. The solidified material is then separated into small pieces (1 mm) and vacuum-dried. Several factors were later found to improve the quality of the microcapsules including the dextrose equivalent of the corn syrup, emulsifier and flavour oil content and emulsification pressure (Crocker & Pritchett, 1978). The advantage of extrusion is that the material is totally isolated by the wall material and that any core is washed from the outside. It is mainly used for visible flavour pieces, vitamin C, colours and extension of shelf-life up to at least 2 years. Dry food applications include drink, cake, cocktail and gelatin dessert mixes since the encapsulated materials are soluble in hot or cold water. Numerous flavours have also been encapsulated by this method (Risch, 1988).

Fluidized bed coating

Solid particles are suspended in a temperature and humidity-controlled chamber of high-velocity air where the coating material is atomized (DeZarn, 1995). Optimal results are obtained with particle sizes between 50 and 500 microns. Particle size distribution should also be narrow. The amount of material that coats the particles is dependent on the length of time that the particles are in the chamber. This technique is applicable for hot-melt coatings such as hydrogenated vegetable oil, stearines, fatty acids, emulsifiers and waxes or solvent-based coatings such as starches, gums, maltodextrins. For hot melts, cool air is used to harden the

carrier, whereas for solvent-based coatings, hot air is used to evaporate the solvent. Hot-melt ingredients release their contents by increasing the temperature or physical breakage, whereas water-soluble coatings release their contents when water is added. Fluidized bed encapsulation can be used to isolate iron from ascorbic acid in multivitamins and in small tablets such as children's vitamins. Many fortified foods, nutritional mixes and dry mixes contain fluidized bed-encapsulated ingredients. Citric acid, lactic acid, sorbic acid, vitamin C, sodium bicarbonate in baked goods, and salt added to pretzels and meats are all encapsulated.

Liposome entrapment

One type of capsule with more versatile properties and less fragility than those made of fat are liposomes. They have been used for delivery of vaccines, hormones, enzymes and vitamins in to the body (Gregoriadis, 1984). They consist of one or more layers of lipids and thus are non-toxic and acceptable for foods. Permeability, stability, surface activity and affinity can be varied through size and lipid composition variations. They can range from 25 nm to several microns in diameter, are easy to make and can be stored by freeze-drying. Kirby and Gregoriadis (1984) have devised a method to

encapsulate at high efficiency which is easy to scale-up and uses mild conditions appropriate for enzymes.

Phospholipids make up the outer layer or layers of liposomes (Figure 2A). The hydrophilic portion of the lipids is oriented towards the aqueous phase and the hydrophobic groups associate with the hydrophobic ones of other lipid molecules. Folding of the lipid sheet into a spherical shape forms a very stable capsule due to there being no interaction of the lipids with water (Figure 2B). Aqueous or lipid-soluble materials, but not both, are entrapped in these membranes. Mainly flavour agents are encapsulated in this manner. Liposomes can range from a few nanometers to micron. They were initially developed for medical purposes (New, 1990) and then were used for cosmetics (Ghychy & Gareiss, 1993). Food applications of liposomes in cheese-making were described by Kirby (1993).

The most common phospholipid in lectin, phosphatidyl choline, is insoluble in water and is inexpensively isolated from soy or egg yolk. The composition of the phospholipids and the process used determine if a single or multiple layers are formed (Martin, 1990). Fatty acids also make up liposomes and their degree of saturation is dependent on the source. Animal

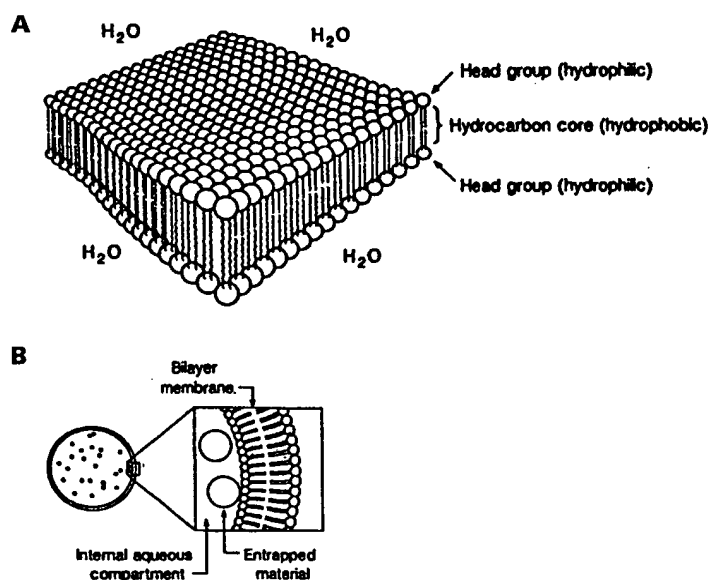


Figure 2. Schematic diagram of a sheet of lipid bilayer (A) and the liposome formed from the lipids (B) (adapted from Reineccius, 1995).

sources provide more saturated fatty acids. They influence the transition temperature which is the conversion from a gel to the more leaky liquid form.

Although sugars and large polar molecules cannot permeate through a liposome bilayer, small lipophilic molecules can. They will only permeate through the membrane, though, if they are soluble in the outside liquid. Hydroxyl ions, protein, and molecules potassium ions permeate very slowly.

Liposomes are made by three different procedures. The lipid formulation is mixed with a solvent system such as 2 : 1 chloroform : methanol. The volume of solvent is decreased and the film of lipids/solvent is then redispersed in an aqueous phase. This step forms the liposomes and it can be done in different ways including physical, two-phase and detergent solubilization. The liposomes are then recovered from the water (New, 1993).

The phospholipids in the liposomes oxidize or hydrolyze over time. Maximum stability can be ensured by using freshly prepared lipid and solvents to prepare the liposomes, avoiding exposure of the liposomes to oxygen as much as possible, limiting excessive temperatures, adding antioxidants and metal chelators to avoid charge neutralization by metals and using proper storage conditions. Hydrolysis can be minimized by using pure solvents and removing as much of the water as possible.

Holding the temperature above the phase transition temperature helps to avoid annealing or fusion. Liposomes smaller than 40 nm are more likely to fuse than larger ones. Since neutral liposomes will still aggregate due to van der Waals forces, addition of 5% phosphatidic acid or phosphatidyl glycerol can reduce this.

Coacervation

National Cash Register Company patented this technique for carbonless paper in the 1950s (Risch, 1995). Particle sizes of a few sub-microns to a centimeter are obtained. Food-grade materials have only recently been used as the carrier. This method, although efficient, is expensive. It consists of dissolving a gelling protein, followed by emulsification of a material such as a flavour oil into the protein. The coating in liquid form is removed from a polymer solution, coats the material to be encapsulated, solidified and collected by cen-

trifugation or filtration. Drying can be accomplished by spray or fluidized bed drying. The factors, pH, temperature and composition are all important in making the microencapsules.

Coacervation can be simple with only one colloidal solute such as gelatin, or complex, with, for example, gelatin and gum acacia (Luzzi & Gerraughty, 1964). Gelatin and gum acacia are used together since at low pH, each has an opposite charge, causing attraction and the formation of an insoluble complex. This viscous solution is more common and can be used to coat flavour oil droplets suspended in an aqueous medium (Bakan, 1969). Lowering the temperature hardens the wall material but it can be softened again by addition of bases, acids, heat or dilution. This process is irreversible if divalent salts or aldehydes are added.

Hydrophilic coatings such as gelatin can be used to microencapsulate hydrophobic substances including citrus or vegetable oils or vitamin A. Hot water, pressure or chemical reaction can be used to release the contents. The coating can also be hydrophobic and the core may be water soluble or immiscible (Balassa & Fanger, 1971).

Inclusion complexation

In this technique, beta-cyclodextrin is used since the centre is hydrophobic while the outer surface is hydrophilic due to its seven glucose units linked 1 to 4. In the centre of the cyclodextrin, water molecules are replaced by less polar molecules (Risch, 1995). The complex then precipitates out of solution (Reineccius & Risch, 1986). Only water can serve as the suspension medium. The precipitate is recovered and dried by conventional means. Binding by the cyclodextrin can occur up to 200°C. The moisture and temperature conditions of the mouth, however, allow release of the bound material.

Garlic and onion oils can be complexed as odour less compounds by cyclodextrin. Vitamins A, E and K which are fat-soluble can also be stabilized in this manner. Cyclodextrin, however, is only approved for use with foods in Japan and Eastern Europe (Dziezak, 1988).

Rotational or centrifugal suspension separation

The steps in rotational suspension separation, which is a relatively new technique (Sparks,

1989), involve mixing the core and wall materials and then adding to a rotating disk. The core materials then leave the disk with a coating of residual liquid. The capsules are then dried or chilled after removal from the disk. The whole process can take between a few seconds to minutes. Solids, liquids or suspensions of 30 microns to 2 mm can be encapsulated in this manner. Coatings can be 1 to 200 microns in thickness and include fats, polyethylene glycol (PEG), diglycerides and other meltable substances. Since this is a continuous, high-speed method that can coat particles, it is highly suitable for foods. One application is to protect foods that are sensitive to or readily absorb moisture such as aspartame, vitamins or methionine (Sparks *et al.*, 1993).

Types of encapsulated food ingredients

The types of food ingredients (Kirby, 1991) that can be encapsulated are shown in Table 1. Most of the uses of encapsulation in foods are for masking odours or tastes. The capsules are usually water-soluble and are dissolved when water is added. Flavour oil encapsulated in a food-grade hydrocolloid is such an example. Microencapsulation also enables ingredients such as enzymes to maintain their viability for extended periods of time as shown in Figure 3. Addition of enzymes unprotected to foods exposes them to ions, protons, radicals, inhibitors, etc. that cause instability and inactivity.

Table 1. Various food ingredients that have been encapsulated

Type of ingredient
Flavouring agents such as oils, spices, seasonings and sweeteners
Acids, alkalies, buffers
Lipids
Redox agents (bleaching, maturing)
Enzymes or microorganisms
Artificial sweeteners
Leavening agents
Antioxidants
Preservatives
Colourants
Cross-linking and setting agents
Agents with undesirable flavours and odours
Essential oils, amino acids, vitamins and minerals

Source: Adapted from Kirby (1991).

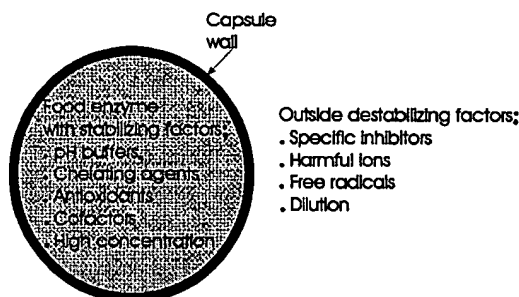


Figure 3. Benefits of encapsulating enzymes in the food industry.

The capsule can shield the enzyme from these factors.

Acidulents are added to processing and preservation aids, and flavour modifiers. Since they interact with gums, starches, proteins and pectins, they can develop a wide range of textures. Encapsulation of these agents can increase the shelf-life of citrus flavours and starch-containing foods and prevent loss of flavour and colour since their release is controlled (Dziezak, 1988). Hygroscopicity and dusting can also be reduced.

Adipic, fumaric, citric, lactic and ascorbic acids have all been encapsulated. Ascorbic acid is added to bread to improve its quality. The encapsulated form can protect this acid from the water and oxygen in the bread which causes degradation (Ozieczak, 1988). Citric acid is added to tea (Dziezak, 1988) to increase tartness but it can react with the tannins and cause discolouring of the tea bag. Encapsulation can avoid this problem while maintaining the function of the citric acid. In cured meats such as pepperoni, hard salami and summer sausages, lactic and citric acids enhance the flavours of these meats. Usually this is accomplished by fermentation which is hard to control. Direct addition is not an option since the acids react with the foods. An alternative is to use encapsulated acids. Bielski (1988) found that production times of cured ground beef are significantly reduced. Glucono-delta-lactone (GDL) is used to cure meats. Encapsulation with fat avoids premature acidity and meat stiffening, and bypasses the fermentation step. Other potential applications include desserts, baking mixes and pet foods.

Beta-carotene, turmeric and other natural colours are not very soluble and can cause dust

problems during handling. The advantages of encapsulating these materials include: extending shelf-life from 6 months to 2 years (Lanzoff, 1988), easier handling, improved solubility and stability.

Encapsulation of citrus oils, other flavouring agents and spices enhance stability. Menthol, peppermint, spearmint, and other flavours in their encapsulated forms are gaining popularity in microwavable and extruded foods because of their stability at high temperatures for short periods of time. Fat-encapsulated cinnamon does not allow this flavour to interfere with yeast growth in baked goods.

Sodium bicarbonate used as a leavening agent can be encapsulated to reduce its reaction with acid or water and provide uniform performance. Fat and oil coatings are typical to encapsulate leavening agents in pizza doughs.

The advantage of encapsulating sodium chloride with partially hydrogenated vegetable oil is to increase ability to flow and reduce clumping and caking. Sodium chloride decreases colour degradation, rancidity, and helps to control water absorption and the growth of yeast. This is particularly applicable for yeast doughs, pretzel snacks and pulverized meats.

Sweeteners can be degraded by temperature and moisture. Sugar and the artificial sweetener, aspartame, is encapsulated with fats in chewing gum. These sweeteners are released slowly during chewing and moisture in the mouth. Aspartame (NutraSweet) can be protected from high temperatures in baking goods by encapsulation. Sweetness would normally be lost as the aspartame breaks down to aspartic acid and phenylalanine (Gibbs *et al.*, 1996).

Vitamins and minerals are usually added to breakfast cereals, dairy products, infant and pet foods. By encapsulating both water and fat-soluble vitamins, off flavours can be avoided and stability can be increased. Flow properties are also enhanced.

Materials of encapsulation

The use of gum arabic as an encapsulating matrix is common due to its characteristics of viscosity, solubility and emulsification. Risch and Reineccius (1988) have reported on the encapsulation of orange oil. Its main disadvantage is its expense due to frequent shortages. Therefore other materials are being investigated. Since

starch derived from potatoes, corn, wheat, rice and others is very plentiful, its derivatives could be used for encapsulation. Unmodified starch is too viscous when mixed with water.

Dextrin is formed by the heating of dry starch with acid or base, forming highly branched polymers. Different products can be obtained depending on the conditions utilized. Compared to unmodified starch, water solubility and viscosity is improved; however, they are unsuitable for oil-based ingredients due to their contribution to flavour and colour.

Starches can also be reacted with 1-octenylsuccinic anhydride to form amphilic groups. The concentration of this agent is limited by law to 3% of the starch (US Code of Federal Regulations). The formation of hydrophilic groups enables encapsulation of lipids almost as well as gum arabic. There has been some evidence, though, that the shelf-life of citrus oils encapsulated in this manner is inferior to gum arabic (Westing *et al.*, 1988).

Maltodextrins are formed by partially hydrolysing corn starch with acids or enzymes, whereas corn syrup solids are dried glucose syrups. Both contain glucose polymers of various lengths. The molecular weight of 10 DE (dextrose equivalent) is approximately 1800 daltons. Their viscosities are lower than gum arabic and they have no lipophilic groups. Therefore their emulsification properties are poor. Their advantages include low flavour, use at high solids concentrations and improvement of the shelf-life of citrus oils.

Blending of corn syrup solids, maltodextrins and modified starches may lead to optimal encapsulating materials. Spray-drying and extrusion processes of the individual components has been used as water-soluble coatings.

Alginates are hydrocolloids extracted from kelp which can react with calcium ions and form a stable gel. They can then be used to entrap or encapsulate flavour oils at ambient temperatures (King, 1983). The alginates are polymers of 12,000 to 180,000 molecular weight composed of D-mannuronic acid and L-guluronic acid connected by 1-4 glycosidic linkages. To make the beads, alginate is emulsified with the flavour oil and then added dropwise to a calcium chloride solution. The bead can be of 200 to 5000 microns in size. Molecules greater than 5,000 daltons are retained by the gels.

Protein-based materials such as polypeptone, soy protein, milk-derived and gelatin derivatives are able to form stable emulsions with volatile flavourings. However, their solubilities in cold water, the potential to react with carbonyls, and their high cost limit potential applications (Bangs & Reineccius, 1988). Other materials such as cyclodextrin and lipid components (liposomes) have already been mentioned.

Methods of release of ingredients from capsules

The release of components can be diffusionally controlled either by the capsule wall or by a membrane covering the wall. The former is called matrix controlled and the latter, membrane controlled. The permeability through the matrix and the solubility of the component of the capsule wall influence the rate of diffusion. In general, the compound to be diffused should be soluble in the matrix. However, this is not necessarily the case, since the vapour pressure of a volatile substance on each side of the matrix can become the major driving force influencing diffusion. The volatility of aromas can vary substantially. For example, octanol has a vapour pressure of 0.18 mm compared to methyl acetate which is 170 mm.

Selection of an appropriate matrix or membrane is thus very important. Chemical nature,

morphology and glass transition temperature, all influence diffusion. However, the selection is limited since food safety is an additional consideration. Less information is available to the food scientist since few databases exist regarding food-compatible matrices or membranes. The degree of swelling is controlled by water absorption or presence of solvents such as glycerin or propylene glycol. The higher water activity, the faster the rate of release. Cross-linking also influences diffusion and is possible by coacervation. Thies (1992) discussed the use of glutaraldehyde as a cross-linker in coacervates. Higher degrees of cross-linking decrease release rates. Aroma release into bulk containers of dry drink mixes of chewing gums is a possible application. Further work using coacervates should be evaluated.

Pressure-activated release has been used for carbonless paper and scratch-and-sniff cards but has not been used frequently for food applications. Aromas could be released by opening a jar. Another application has been developed by Parliament *et al.* (1989) where a package is microwaved, heated and releases aromas during the process.

The most commonly used method of controlled release in the food industry is solvent-activated. Flavour is released from dry products such as dry beverages or cake mixes as water is added. Coatings based on sugars, gelatin, starches, PEG and others are used. In these cases,

Table 2. Encapsulation techniques used in chewing gum

Attribute	Encapsulation technique			
	Extrusion	Spray drying	Fluidized bed	Coacervation
Acidulent				
Long lasting	×		×	
Few reactions with colour	×	×	×	
Low sucrose inversion	×	×	×	
Flavour				
Immediate	×	×		×
Delayed			×	
Long-lasting				×
High concentration				×
Sweetener				
Immediate	×	×		
Delayed	×		×	
Sustained release	×		×	
Temperature	×	×	×	

Source: Adapted from Cherukuri (1992).

release is immediate, unlike for chewing gum where release over a long period of time is preferred. Other components such as sweeteners and acidulents must also be released from the chewing gum gradually. Numerous patents have been obtained. Various techniques are used as shown in Table 2.

One method that is used to control the release of flavours in chewing gum is to perform an initial spray-drying step and then coat the particle with a gum, wax or other water-insoluble substances. Acidulents are encapsulated for slow release and to avoid reactions with colours and sugars. Sweeteners such as aspartame or acesulfame K are encapsulated with an extra fatty or waxy coating to add stability (Song, 1990) and allow release over an extended period of time. As shown in Table 1, several techniques are used. One difficulty with this method, though, is that the flavour concentration is diluted by addition of the additional coating which can make up to 50% of the weight of the capsule. Higher concentrations of flavour must be used but this can change the flow properties of the gum. Release of enzymes through a change in pH is possible with liposomes (Karel & Langer, 1988) which can be destabilized. A preservative, sorbic acid, has been concentrated at the surface at a different pH than the bulk solution by mixing with an anionic polyelectrolyte, carrageenin. This can extend the shelf life of foods from hours to weeks.

Another mechanism of release is by melt-activation. The membrane on the wall or the wall itself made of lipids or waxes is destroyed by melting and components such as salts, leavening, flavourings and nutrients are released. Spray chilling is frequently used in this case. This technique is limited to water-soluble flavourings since those which are hydrophobic will pass through the wall material. Low-viscosity coatings are useful to release products upon stirring.

The release of the enzyme from the enzyme/substrate complex can be site-specific, time/stage-specific or signalled by changes in pH, temperature, irradiation or osmotic shock. Alteration of the surface properties of the microcapsule is performed so that the capsules will accumulate at a certain location for release at the specific location. Selection of more-stable microcapsules will delay the release of the

enzyme. Microcapsules made of hardened fats are insoluble in water and can release the contents when subjected to shear or increased temperature which melts the fat. This type is widely used in soup mixes, bakery products or high-fat products (Dziezak, 1988).

Other materials such as the antioxidant *butylated hydroxytoluene* (BHT), however, are encapsulated to improve handling. Normally, it is very sticky but encapsulation with methyl cellulose enables it to be easily added to fatty materials which then dissolve the capsule wall and release the antioxidant. Studies (Karel & Langer, 1988) have also indicated that ultrasonics and surfactants (e.g. Triton X-100) can also induce enzyme release in cheese-ripening from microcapsules such as liposomes. Chewing can release flavours such as herbs or garlic in pizza which are encapsulated in 1000 micron particles.

Applications of liposomes

Liposomes are being developed for use in cheese-making for reducing ripening time and preventing spoilage (Figure 4). Addition of protease enzymes is commercially used in the United States and other countries since ripening times can be reduced from a year to half a year. The use of liposomes would allow the enzymes to be dispersed uniformly in the milk and avoid the brine environment in hard cheeses which is normally too harsh for the enzymes. The liposomes are added to the milk after coagulation and will break down within the next few hours, releasing the enzymes (Kirby & Law, 1986).

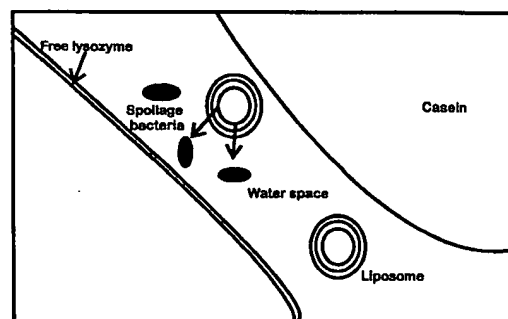


Figure 4. Microencapsulation of lysozyme in liposomes to prevent spoilage in cheese by bacteria (adapted from Kirby, 1991).

Cheeses such as Gouda, Edam and Emmental can be spoiled by butyric acid fermenting bacteria. Nitrate can be used to control this problem but there are some health concerns. Liposomes can be used to encapsulate the enzyme, lysozyme (Thapon & Brule, 1986) or the antibiotic, nisin, which could be used to prevent spoilage by bringing these components to the areas where spoilage is most likely.

Another promising area for liposomes is the prevention of oxidation of unsaturated fats in food emulsions such as spreads, margarines or mayonnaise. This is a new application since saturated fats are now being replaced by unsaturated ones which are susceptible to oxidation. Natural anti-oxidants are preferred since many synthetic varieties are banned. One approach is to entrap Vitamin C in the interior and alpha-tocopherol (Vitamin E) in a liposome layer (Kirby, 1990). Currently lipid-soluble, chemical derivatives of vitamin C are used.

Encapsulation patents

Recent developments in the encapsulation of foods have been mainly in the areas of controlled release, carrier materials, encapsulation methods and sweetener encapsulation. Most patents are concerned with controlled and sustained release. Their main objective is to lead to new and improved products. For example, the release of flavours and sweeteners from chewing gum has received considerable attention. International Flavours and Fragrances (IFF) developed a mixture of polyethylene and polyethylene glycol to encapsulate and provide sustained release of 2-methyl-2-pentenoic acid, the strawberry flavour in gum (Rutherford *et al.*, 1992). Wm Wrigley Jr Co. and Warner-Lambert Company have been actively involved in the development of chewing gums. Wrigley obtained a patent that concerned mixing sweetener with a wax coating (Zibell, 1989), incorporation of the encapsulated sweetener Alitame into the liquid part of gum (Song, 1990) and many other patents.

Since aspartame is sensitive to heat, methods to protect it are being developed. Encapsulation with ethyl- or methylcellulose (Redding *et al.*, 1992) or a mixture of lecithins, fatty acids, waxes, glycerides and an anti-foaming agent (Bodor & Dokuzovic, 1992) have been examined. Nabisco Brands has developed processes

for liposomes. The liposome capsules are added to water, flour and shortening at 150°F in an extruder so that the liposomes remain intact to provide the required dough texture upon baking (Finley *et al.*, 1991), while another application involves encapsulation of an unsaturated lipid by liposomes into margarine (Haynes *et al.*, 1992).

Conclusions

Numerous developments have been made in the field of encapsulated food ingredients. Manufacturing techniques include spray drying, spray chilling or spray cooling, extrusion coating, fluidized bed coating, liposome entrapment, coacervation, inclusion complexation, centrifugal extrusion and rotational suspension separation. There are many requirements for the controlled and sustained release of food ingredients. New markets will be developed as advances in encapsulation continue. Coacervation seems to be particularly promising since the cost can be reduced due to the requirement for lower levels of food ingredients. In addition, flavours are more stable after processing with microwave, heat, oven drying and frying.

Limitations in many of the encapsulation techniques have occurred due to high costs of production and the lack of food-grade available materials. Research is necessary to eliminate these limitations. Encapsulation currently is an art that is difficult for the food scientist to master. The food scientist does not have the information available in databases to enable him to make informed choices concerning the most appropriate material and encapsulation process. For example, the appropriate blends of starches and maltodextrins as encapsulating materials could prove highly beneficial. The development of cyclodextrins has led to new products with longer shelf-life, reduced volatility and protection of heat-labile substances.

Preliminary indications are that liposomes have many benefits for the food industry including protection of materials until desired release or targeted delivery. There is a great deal of research that needs to be done concerning the use of liposomes in the food industry. Unlike the pharmaceutical industry, which can tolerate high costs, manufacturing costs will have to be reduced for food applications.

References

- Bakan JA (1969): US Patent No. 3,436,355, National Cash Register.
- Balassa LL & Fanger GO (1971): Encapsulation in the food industry. *CRC Rev. Food Technol.* **2**, 245.
- Bangs WE & Reineccius GA (1988): Corn starch derivatives. In *Flavor Encapsulation*, eds SJ Risch & GA Reineccius, pp. 12–28. ACS Symposium Series No. 370. Washington, DC: American Chemical Society.
- Bhandari BR, Dumoulin HMJ & Richard HMJ (1992): Flavor encapsulation of spray drying: application to citral and linalyl acetate. *J. Food Sci.* **51**, 1301–1306.
- Blenford D (1986): Fully protected. *Food Flavor Ingrid. Proc. Pckg.* July, p. 43.
- Bodor Z & Dokuzovic Z (1992): Encapsulation matrix. US Patent No. 5,126,151.
- Chang TMS (1978): *Artificial Kidney, Artificial Liver and Artificial Cells*. New York: Plenum.
- Cherukuri SR (1992): Flavor/taste controlled release and improved stability. Second Workshop on the *Controlled Delivery in Consumer Products*, Controlled Release Society, 13–15 May, Secaucus, NJ.
- Crocker GC & Pritchett DE (1978): Improved encapsulated citrus oils. *Food Technol.* **32**, 36–45.
- Desoize B (1986): In vitro cytotoxic activity of cross-linked protein microcapsules. *J. Phar. Pharmacol.* **38**, 8–13.
- De Zarn TJ (1995): Food ingredient encapsulation. In *Encapsulation and Controlled Release of Food Ingredients*, eds SJ Risch & GA Reineccius, pp. 74–86. ACS Symposium Series 590. Washington, DC: American Chemical Society.
- Dziezak JD (1988): Microencapsulation and encapsulated food ingredients. *Food Technol.* **42**, 136–151.
- Finley J, Haynes LC, Lengerich BV, Levine H, Mathewson P & Otterburn MS (1991): Extrusion baking of cookies using liposome encapsulated ingredients. US Patent No. 4,999,208.
- Ghychy M & Gareiss J (1993): Industrial preparation of liposomes for cosmetic products. Presented at a *Workshop on the Controlled Delivery of Consumer Products*, Controlled Release Society, 21–23 October, Geneva.
- Gibbs BF, Alli I & Mulligan CN (1996): A simple and rapid HPLC method for the determination of aspartame and its metabolites in foods. *J. Chrom.* **725**(2), 372–377.
- Gregoriadis G (ed.) (1984): In *Liposome Technology*, vols 1–3. Boca Raton, FL: CRC Press.
- Haynes LC, Levine H & Finley JW (1992): Method and liposome composition for the stabilization of oxidizable substances. US Patent No. 5,139,803.
- Karel M & Langer R (1988): Controlled release of food additives. In *Flavor Encapsulation*, eds SJ Risch & GA Reineccius, pp. 177–191. ACS symposium Series No. 370. Washington, DC: American Chemical Society.
- King AH (1983): Brown seaweed extracts (alginates). In *Food Hydrocolloids*, ed. M Glicksman, vol. 2, ch. 6. pp. 115–188. Boca Raton, FL: CRC Press.
- Kirby CJ (1990): Delivery systems for enzymes. *Chemistry in Britain* **26**, 847–850.
- Kirby CJ (1991): Microencapsulation and controlled delivery of food ingredients. *Food Sci. Technol.* **5**, 74–78.
- Kirby CJ (1993): Controlled delivery of functional food ingredients: opportunities for liposomes in the food industry. In *Liposome Technology*, 2nd ed. Vol. 2: *Entrapment of Drugs and Other Materials*, ed. G Gregoriadis, pp. 215–232. Boca Raton, FL: CRC Press.
- Kirby CJ & Gregoriadis G (1984): A simple procedure for preparing liposomes capable of high encapsulation efficiency under mild conditions. In *Liposome Technology*, vol. 1, ed. G Gregoriadis, pp. 19–27. Boca Raton, FL: CRC Press.
- Kirby CJ & Law BA (1986): Recent developments in cheese flavor development technology: application of enzyme encapsulation. *Proceedings of an Online Conference on Biotechnology in the Food Industry*, pp. 17–22. London: Online Publications.
- Larisch B (1990): Microencapsulation of *Lactococcus lactis* subsp. *cremoris* for application in the dairy industry, MSc thesis, McGill University, Montreal, Canada.
- Lee BH (1996): *Fundamentals of Food Biotechnology*, New York: VCH Publishers.
- Linko P (1985): Immobilized lactic acid bacteria. In *Enzymes and Immobilized Cells in Biotechnology*, ed. A Larson, pp. 25–36. Minto Park, CA: Benjamin Cummings.
- Luzzi LA & Gerraughty RJ (1964): Effects of selected variables on the extractability of oils from coacervate capsules. *J. Pharm. Sci.* **53**, 429–435.
- Martin FP (1990): Pharmaceutical manufacturing of liposomes. In *Specialized Drug Delivery Systems*, ed. P Tyle, pp. 50–57. New York: Marcel Dekker.
- New RRC (1990): Introduction. In *Liposomes, a Practical Approach*, ed. RRC New, pp. 1–32. New York: IRL Press.
- New RRC (1993): Preparation of liposomes. In *Liposomes, a Practical Approach*, ed. RRC New, pp. 33–104. New York: IRL Press.
- Parliment TH, Cipriano JJ & Scapellino R (1989): Aroma release during microwave cooking, US Patent No. 4,857,340.
- Redding BK Jr, Butcher B, Garrison WS & Schmucker AE, (1992) PCTInt. Appl. WO 9211084.
- Reineccius GA (1995): Liposomes for controlled release in the food industry. *Encapsulation and Controlled Release of Food Ingredients*, eds SJ Risch & GA Reineccius, pp. 113–133. ACS Symposium Series 590. Washington, DC: American Chemical Society.
- Reineccius GA & Risch SJ (1986): Encapsulation of artificial flavors by beta cyclodextrin. *Per. Flav.* Aug/Sept., pp. 1–11.
- Reineccius GA, Ward FM, Whorton C & Andon SA (1995): Developments in gum acacias for the encapsulation of flavors. In *Encapsulation and Controlled Release of Food Ingredients*, eds SJ Risch & GA Reineccius, pp. 161–168. ACS Symposium Series 590. Washington, DC: American Chemical Society.
- Risch SJ (1988): Encapsulation of flavors by extrusion. In *Flavor Encapsulation*, ACS eds SJ Risch & GA Reineccius, pp. 103–109. ACS Symposium Series 370. Washington, DC: American Chemical Society.
- Risch SJ (1995): Encapsulation: overview of uses and techniques. In *Encapsulation and Controlled Release of Food Ingredient*, eds SJ Risch & GA Reineccius, pp. 1–7. ACS Symposium Series 590. Washington, DC: American Chemical Society.
- Risch SJ & Reineccius GA (1988): Spray-dried orange oil: effect of emulsion size on flavor retention and shelf

- stability In *Flavor Encapsulation*, eds SJ Risch & GA Reineccius, pp. 67-77. ACS Symposium Series 370. Washington, DC: American Chemical Society.
- Rutherford HJ, Desai N, McDermott K & Wiener C (1992): Chewing gum containing composition for controlled release of flavor bearing substances and process for producing same. US Patent No. 5,116,627.
- Seiss W & Divies C (1975): Microencapsulation. *Angew. Chem. Int. Ed.* 14, 539-550.
- Song JH (1990): Gradual release structures for chewing gum. US Patent No. 4,978,537.
- Sparks RE (1989): Microencapsulation. In *Encyclopedia of Chemical Process Technology*, ed. J McKetta. New York: Marcel Dekker.
- Sparks RE, Mason NS, Autant P, Cartiller A & Pigeon R (1993): Composition for feeding ruminants. US Patent No. 5,186,937.
- Swisher HE (1957): US Patent No. 2,809,895.
- Taylor AH (1983): Encapsulation systems and their applications in the flavor industry. *Food Flavol. Inged. Proc. Pckg.*, Sept., pp. 48-52.
- Thapon JL & Brule G (1986): Effets du pH et de la forme ionize sur l'affinité lysozyme-caseines. *Le Lait* 66, 19-30.
- Thevenet F (1995): Acacia gums: natural encapsulation agent for food ingredients. In *Encapsulation and Controlled Release of Food Ingredient*, eds SJ Risch & GA Reineccius, pp. 51-59. ACS Symposium Series 590. Washington, DC: American Chemical Society.
- Thies C (1992): Encapsulation processes for controlled delivery applications. Second Workshop on the *Controlled Delivery in Consumer Products*, May 13-15, Controlled Release Society, Secaucus, NJ.
- US Code of Federal Regulations. *Title 21*. Washington, DC: U.S. Government Printing Office.
- Westing LL, Reineccius GA & Caporaso F (1988): Shelf-life of orange oil: Effects of encapsulation by spray-drying, extrusion and molecular inclusion: eds SJ Risch & GA Reineccius, pp. 110-121. ACS Symposium Series 370. Washington, DC: American Chemical Society.
- Zibell SE (1989): Method of making chewing gum with wax-coated delayed release ingredients. US Patent No. 4,885,175.